Sociology & Economics
Detroit Public Library

U.S. 96
P9N1c13
v.61, no.7

cancer treatment reports

Detroit Public Library
NOV 1 [illegible]
Depository Document

Volume 61
Number 7
October 1977
Pages 1231-1417

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service National Institutes of Health

cancer treatment reports

National Cancer Institute, Guy R. Newell, Jr., *Acting Director*

Division of Cancer Treatment, Vincent T. DeVita, Jr., *Director*

Editorial Board

Bruce A. Chabner, *Editor-in-Chief*
Associate Editors:
Paul A. Bunn
Michael Colvin
David A. Cooney
John S. Driscoll
Marc E. Lippman
John S. Macdonald
Stephen C. Schimpff
Susan M. Sieber
John M. Venditti
John L. Ziegler

Editorial Staff

Pamela W. Hutchins, *Managing Editor*
Jean M. Griffin, *Assistant Editor*
Elaine P. Beck, *Assistant Editor*
Virginia T. Ascosi, *Editorial Assistant*
Florence L. Barnes
Mary M. Davis
Deidre A. Hoehn

Advisory Board

Bernard Fisher
Emil J Freireich
Lewis W. Guiss
Simon Kramer
Faith H. Kung
LaSalle D. Leffall, Jr.
John A. Montgomery
David G. Nathan
Paul E. Neiman
Stanley E. Order
Theodore L. Phillips
Philip Rubin
Edward F. Scanlon
Stuart F. Schlossman
Joseph V. Simone

Editorial Policy

The Editorial Board will consider manuscripts dealing with all aspects of preclinical and clinical cancer treatment. These may include such topics as surgery, radiotherapy, chemotherapy, immunotherapy, supportive care, pharmacology, cell biology and kinetics, mechanisms of drug action, and medicinal chemistry.

The journal will consist of two parts. One part will consider previously unpublished manuscripts of original work under four main categories:

1. Clinical or preclinical research.
2. Correspondence regarding manuscripts previously published in *Cancer Treatment Reports*. Letters on other topics will be published at the discretion of the Editorial Board.
3. Invited guest editorials and reviews. Unsolicited contributions will be considered on their individual merits.
4. Brief communications. Under this category the Editorial Board will consider short contributions which should not exceed five typewritten pages. Four types of communication will be considered in this category.
   (a) Ideas relating to cancer treatment.
   (b) Preliminary research reports.
   (c) Clinical case reports.
   (d) Brief clinical trials of novel treatments.

All material submitted for consideration in the journal will be subject to review when appropriate by at least two outside reviewers and one member of the Editorial Board. The decision to publish a manuscript is solely the responsibility of the Editorial Board.

The second part of this journal will consist of supplements (published bimonthly) which may include proceedings of meetings and symposia and program information from the Division of Cancer Treatment, National Cancer Institute. Requests to publish information in *Cancer Treatment Reports* supplement will be considered by the Editorial Board.

INSTRUCTIONS TO AUTHORS

Summary
Begin the article with a short summary.

Text
Submit an original and three copies of the manuscript, typed double spaced. Each page should begin with a new paragraph. Use accepted spelling, abbreviation, and hyphenation found in *Webster's New International Dictionary*. Footnotes should be typed at the bottom of the page on which they appear.

Tables
Submit each table on a separate page, typed double spaced. Please use standard abbreviations; otherwise spell out.

References
Submit references typed double spaced. Follow the standard form of *Index Medicus:* author, title, name of journal, volume number, page or inclusive pages, and year. In book references give author, title of book, edition (if more than one), city, name of publisher, date of book, volume number, and pages cited. References in text should begin with number 1 and continue consecutively. References to papers presented at meetings should include author; title of paper; title, city, and date of meeting; city and name of publisher; date of publication; volume number; and pages. Papers presented at meetings but not published should be cited in a footnote rather than in the reference section.

Illustrations
Photographs and graphs preferably should be clear, glossy prints. Original art work should be done in black ink for good reproduction. Please send 2 copies of each print.

Nomenclature
Names of compounds should conform to American usage. See *Chemical Abstracts* for proper nomenclature.

Abbreviations
Only standard abbreviations are acceptable. If a new or coined abbreviation is used, it must be defined when first mentioned.

Brief Reports
Manuscripts published in the Brief Reports section can be no longer than 5 double-spaced pages including references and tables. Summaries and figures are usually omitted.

Symposia
Requests to publish the proceedings of symposia should be addressed to the Managing Editor. A copy of the agenda and a list of speakers should accompany such requests.

Submission of Manuscripts
Manuscripts and all inquiries concerning the status of manuscripts should be addressed to: Managing Editor, Cancer Treatment Reports, National Cancer Institute, 8300 Colesville Rd, Rm 3A05, Blair Bldg, Silver Spring Md 20910. We invite all authors to submit with their manuscripts a short list of possible reviewers. The editorial board will take these names into consideration in selecting those who will be asked to evaluate your manuscript.

Reprints
Supplied free of charge: 250 reprints will be sent to the senior author about three weeks after publication of the article.

SUBSCRIPTIONS

Cancer Treatment Reports is for sale by the Superintendent of Documents, US Government Printing Office, Washington, DC 20402. The yearly subscription rate is $38.35 domestic, with an additional $9.60 for foreign mailing. The prices vary for individual issues. Payment is required in advance; check or money order should be made payable to the Superintendent of Documents. Please specify List ID "CAN" (the identifying code for this journal) when requesting a subscription.

Contents

Cancer Treatment Reports
Vol. 61, No. 7
October 1977

Page

COMMENTARY

Drug Interactions With Antineoplastic Agents 1231
Robert D. Warren and Richard A. Bender
National Cancer Institute, Bethesda, Maryland

ARTICLES

Epipodophyllotoxin VM-26 in the Treatment of Childhood Neuroblastoma 1243
Gaston Rivera, Alexander Green, Ann Hayes, Thomas Avery, and Charles Pratt
St. Jude Children's Research Hospital, Memphis, Tennessee

Implications of the ^{99m}Tc Diphosphonate Bone Scan on Treatment of Primary Breast Cancer 1249
D. L. Citrin, D. C. Tormey, and P. P. Carbone
University of Wisconsin Hospital, Madison

Effects of Adriamycin and Actinomycin D on Nucleolar Morphology: A Simple Biologic Assay 1253
Karel Smetana, Jerome Merski, Yerach Daskal, Rose K. Busch, and Harris Busch
Baylor College of Medicine, Houston, Texas

Murine Myeloid Leukemia: I. Pathophysiology and Drug Sensitivity 1259
Harvey D. Preisler, Sigurdur Bjornsson, and Mayumi Mori
Roswell Park Memorial Institute, Buffalo, New York

Distribution and Metabolism of Vincristine in Mice, Rats, Dogs, and Monkeys 1269
Salah M. El Dareer, Vivian M. White, Francis P. Chen, L. Bruce Mellett, and Donald L. Hill
Southern Research Institute, Birmingham, Alabama

Distribution and Metabolism of Prednisone in Mice, Dogs, and Monkeys 1279
Salah M. El Dareer, Robert F. Struck, Vivian M. White, L. Bruce Mellett, and Donald L. Hill
Southern Research Institute, Birmingham, Alabama

5-Fluorouracil-Induced Increase of Total Serum Thyroxine and Triiodothyronine 1291
L. Beex, A. Ross, A. Smals, and P. Kloppenborg
St. Radboud Hospital, University of Nijmegen, The Netherlands

Page

Localization of *meso*-Tetra(*p*-sulfophenyl)porphine in Murine Sarcoma Virus-Induced Tumor-Bearing Mice 1297
Carl J. Carrano, Minoru Tsutsui, and Stewart McConnell
Texas A and M University, College Station

Evaluation of Radiotherapy for Localized Inflammatory Skin and Perianal Lesions in Adult Leukemia: A Prospectively Randomized Double Blind Study 1301
John A. Levi, Stephen C. Schimpff, Robert G. Slawson, and Peter H. Wiernik
Baltimore Cancer Research Center, National Cancer Institute, Maryland

Tumor Size, Sensitivity to Therapy, and Design of Treatment Schedules 1307
Larry Norton and Richard Simon
National Cancer Institute, Bethesda, Maryland

Differential Distribution of Antitumor Agents in Primary and Secondary Tumors 1319
M. G. Donelli, T. Colombo, M. Broggini, and S. Garattini
Istituto di Ricerche Farmacologiche "Mario Negri," Milan, Italy

Experimental Chemotherapy With 5-Arylpyrimidine Antifolates: Further Studies on Toxicity and the Responsiveness of Ascitic, Solid, and Intracranial Sarcoma 180 to DDMP and DDMP With Citrovorum Factor 1325
F. M. Sirotnak, D. M. Moccio, and D. M. Dorick
Memorial Sloan-Kettering Cancer Center, New York, New York

Acute Toxicity of Maytansine in F344 Rats 1333
G. M. Mugera and J. M. Ward
National Cancer Institute, Bethesda, Maryland

Platinum-Based Polychemotherapy Versus Dianhydrogalactitol in Advanced Non-Small Cell Lung Cancer 1339
Robert T. Eagan, James N. Ingle, Stephen Frytak, Joseph Rubin, Larry K. Kvols, David T. Carr, Douglas T. Coles, and Judith R. O'Fallon
Mayo Clinic, Rochester, Minnesota

Tetrahydrouridine: Physiologic Disposition and Effect Upon Deamination of Cytosine Arabinoside in Man 1347
Willi Kreis, Thomas M. Woodcock, Clara S. Gordon, and Irwin H. Krakoff
Memorial Sloan-Kettering Cancer Center, New York, New York

Combinations of Tetrahydrouridine and Cytosine Arabinoside in Mouse Tumors 1355
Willi Kreis, Catherine Hession, Angela Soricelli, and Kevin Scully
Memorial Sloan-Kettering Cancer Center, New York

BRIEF REPORTS AND PRELIMINARY COMMUNICATIONS

Phase II Trial With Bleomycin, CCNU, and Streptozotocin in Patients With Metastatic Cancer of the Breast 1365
P. R. Band, G. P. Canellos, M. Sears, L. Israel, and S. J. Pocock
Eastern Cooperative Oncology Group

Page

Treatment of Advanced Ovarian Carcinoma With High-Dose Cyclophosphamide After Failure on Melphalan 1369
Leo B. Twiggs and C. Paul Morrow
University of Southern California Medical Center, Los Angeles

Phase II Trial of Asaley in Children With Late-Stage Acute Lymphocytic Leukemia 1373
Charles L. Sexauer, Samuel Morgan, Jan van Eys, and Diane M. Komp
Southwest Oncology Group

Chemotherapy of Metastatic Gastrointestinal Neoplasms With 5-Fluorouracil and Streptozotocin 1375
Mark Seligman, Ronald M. Bukowski, Carl W. Groppe, Jr, James K. Weick, James S. Hewlett, and Richard L. Greenstreet
The Cleveland Clinic Foundation, Ohio

Phase II Study of β-2′-Deoxythioguanosine in Adult Acute Leukemia 1379
G. A. Omura, W. R. Vogler, R. V. Smalley, N. Maldonado, G. O. Broun, W. H. Knospe, Y. S. Ahn, and G. B. Faguet
Southeastern Cancer Study Group

Adriamycin Versus Adriamycin and Bleomycin in Advanced Epidermoid Carcinoma of the Cervix 1383
Bernard R. Greenberg, Carl G. Kardinal, Thomas F. Pajak, and Joseph R. Bateman
Western Cancer Study Group

Adriamycin Therapy in American Patients With Hepatocellular Carcinoma 1385
Daniel C. Ihde, Robert C. Kane, Martin H. Cohen, K. Robert McIntire, and John D. Minna
National Cancer Institute-Veterans' Administration Medical Oncology Branch, Washington, DC

Changes in Glomerular Filtration Rate Associated With High-Dose Methotrexate Therapy in Adults 1389
Stephen B. Howell and Johanna Carmody
Sidney Farber Cancer Institute, Boston, Massachusetts

Effect of Hydration on Plasma-Methotrexate Levels 1393
J. L. Romolo, N. H. Goldberg, K. R. Hande, and S. A. Rosenberg
National Cancer Institute, Bethesda, Maryland

Treatment of Refractory Acute Leukemia With High-Dose Chemotherapy 1397
B. S. Kramer, F. R. Appelbaum, G. P. Herzig, R. G. Graw, Jr, and A. S. Levine
National Cancer Institute, Bethesda, Maryland

Heparin and Polychemotherapy for Treatment of Lung Cancer 1399
Jay J. Rohwedder and Edmundo Sagastume
Veterans' Administration Hospital, Ann Arbor, Michigan

Page

Continuous Pelvic Arterial Infusion With Bleomycin for Squamous Carcinoma of the Cervix Recurrent After Irradiation Therapy 1403
C. Paul Morrow, Philip J. DiSaia, Charles F. Mangan, and Leo D. Lagasse
Gynecologic Oncology Group

Serum Displacing Agents in Cancer Chemotherapy 1407
Fred J. Schiffman, Joyce M. Fisher, and Marco Rabinovitz
National Cancer Institute, Bethesda, Maryland

LETTERS

Lack of Severe Hypotension With VP–16–213 Administered Directly Iv 1411
F. Cavalli, R. W. Sonntag, H. J. Ryssel, and K. W. Brunner
University Clinics, Inselspital, Bern, Switzerland

Time Relationship Between Last Dose of Daunorubicin and Congestive Heart Failure 1411
Daniel D. Von Hoff, Maxwell Layard, Marcel Rozencweig, and Franco M. Muggia
National Cancer Institute, Bethesda, Maryland

Unexpected Pain Syndrome During a Phase I Study of Iv Methyl-CCNU 1413
Charles D. Haas, Ronald L. Stephens, Carol J. Leite, J. Wendall Goodwin, and Barth Hoogstraten
Kansas University Medical Center, Kansas City

ANNOUNCEMENTS 1417

Medical Oncology Fellowships
Faculty Position: Hematology/Oncology
Seminars in Oncology at Roswell Park Memorial Institute

The Secretary of Health, Education, and Welfare has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through January 31, 1980.

COMMENTARY

Drug Interactions With Antineoplastic Agents [1]

Robert D. Warren and Richard A. Bender [2,*]

During the past 25 years, the use of drugs in the treatment of malignancies has resulted in increased survival in many types of cancer and potential cures in others. The utility of single antineoplastic agents and the observation in animals that an enhanced therapeutic effect was achieved when two or more agents were combined led to clinical trials applying combination chemotherapy to large patient populations. The success of these combinations has been reported by many investigators and combination chemotherapy has become the accepted treatment for many malignancies.

Each antineoplastic agent has its own pharmacologic characteristics and side effects and these have recently been reviewed (1). Both the pharmacodynamics and the side effects of one drug may be modified by the concomitant administration of another antineoplastic drug. In addition, patients with malignancies frequently require other medications as well. These medications may alter the efficacy of the chemotherapeutic drugs, and adverse reactions, some of which may be life threatening, can occur.

Reports have emphasized the increasing incidence of possible adverse drug interactions (2), have related this incidence to the number of medications prescribed (3), and have admonished the medical community to avoid multiple-drug therapy whenever possible (4). Specific interactions of antineoplastic agents have received previous attention, but earlier work cites only some of the interactions reported to occur with this class of agents (5–7). This paper summarizes currently known interactions of antineoplastic agents with other nonantineoplastic compounds and relates these interactions to their suspected pharmacologic mechanisms. Reported interactions in man, as well as animal studies which may have clinical significance, will be discussed.

[1]Received Feb 10, 1977; revised June 13, 1977; accepted June 21, 1977.

[2]Division of Cancer Treatment (DCT), National Cancer Institute (NCI), Bethesda, Md.

*_Reprint requests to_: Richard A. Bender, MD, DCT, NCI, Bldg 10, Rm 12N226, National Institutes of Health, Bethesda, Md 20014.

MECHANISMS OF DRUG INTERACTIONS

A drug interaction usually means that the effects of one drug are altered by prior or simultaneous use of another drug. The interaction may be beneficial or adverse. Drug interactions are mediated by changes in gastrointestinal absorption, interaction at plasma protein binding sites, increased or decreased metabolism or renal excretion of the parent drug or its metabolite, interaction at tissue receptor sites, or direct physical or chemical interaction between drugs (4,8,9). Drugs which rely on active transport mechanisms may show altered activity when other agents compete for their transport systems. Finally, drugs may combine to form a new compound or complex with totally different characteristics.

A factor particularly important in drug interactions is the displacement from the plasma protein binding sites of one drug by another (10). This increases the unbound fraction of the displaced drug, providing more pharmacologically active drug to the cellular receptor sites as well as to the metabolic and excretory pathways. This mechanism is especially pertinent to drugs such as methotrexate, which binds readily to serum albumin (10) but can be displaced from albumin by sulfonamides and salicylates with a potential increase in drug toxicity (11).

Administration of one drug can alter the metabolism of another drug previously or concurrently administered. Since metabolism of many drugs, including many antineoplastic agents, is handled by liver microsomal enzymes, any one of the several agents now known to activate these enzymes may alter the activity of a concurrently administered antineoplastic agent. Phenobarbital, a well-known inducer of liver microsomal enzyme systems, has been shown to accelerate production of the nitrobenzyl pyridine-measured alkylating metabolites of cyclophosphamide in mice pretreated with phenobarbital (12). This increased rate of drug metabolism generally results in decreased pharmacologic activity, unless, as in the case of cyclophosphamide, the metabolite of the drug is more active than the

parent compound. Decreased metabolism may occur when drugs inhibit degradative enzymes, either by competitive inhibition of the same enzyme site or by directly blocking the enzyme activity itself. An example of this interaction is the alteration of the plasma half-life and therapeutic effects of 6-mercaptopurine when allopurinol, a xanthine oxidase inhibitor, is administered concurrently (13).

Interactions may also occur at the site of drug excretion. Renal excretion may depend on the glomerular filtration rate, on the degree of ionization of a weak acid or base as it relates to reabsorption across tubular membranes, or on active transport mechanisms at renal tubular sites. Drugs which increase the pH of the glomerular filtrate can decrease reabsorption of a weak acid, such as salicylic acid, while the converse is true for weak bases. This is the case for methotrexate whose major route of excretion is via the kidney, probably by a combination of glomerular filtration and tubular secretion. Methotrexate clearance in man is inhibited by concurrent administration of salicylates, which presumably rely on the same renal transport system for their excretion (14).

Finally, many of the pharmacologic effects of antineoplastic agents, and other agents as well, are determined by reactions which occur at specific receptor sites. This specificity is thought to arise from spatial symmetry between the drug and receptor site. Another drug may also have the appropriate configuration to fit the receptor site and competition may occur when both drugs are present. Since drug transport mechanisms may rely on cellular receptor sites, competition for cellular uptake may also occur.

ALKYLATING AGENTS

Most reported studies of drug interactions with alkylating agents involve cyclophosphamide, especially since its metabolism is altered by pretreatment with, or simultaneous administration of, other agents (table 1). Cyclophosphamide, unlike other alkylating agents, is inactive until converted to 4-hydroxy-cyclophosphamide and aldophosphamide (1). Liver microsomal enzymes are thought to be involved in this process and several investigators using animal systems have observed the effect of pretreatment with barbiturates on the formation of the active metabolites of cyclophosphamide. Hepatic microsomal metabolizing enzymes may vary greatly in activity depending on animal species and the effect of these enzymes on a particular drug interaction may also vary in different animal tumor models, leading to conflicting information. Such conflicting data exist for the phenobarbital-cyclophosphamide interaction. Field and co-workers (12) studied the effects of pretreatment with phenobarbital or 2-diethylaminoethyl-2,2-diphenylvalerate (SKF-525A), an inhibitor of liver microsomal enzymes, on the survival of L1210 tumor-bearing mice treated with cyclophosphamide. The mice were treated at various times prior to and simultaneously with implantation of tumor and mean survival times were used as a criterion for retention of the antileukemic activity of cyclophosphamide. In addition, the active or alkylating metabolites of cyclophosphamide were measured. Pretreatment with phenobarbital accelerated production of the alkylating metabolites, followed by rapid depletion of the parent compound and its metabolites, and resulted in a shorter duration of the antileukemic effect as measured by shortened survival times. SKF-525A pretreatment retarded activation of cyclophosphamide, increased the amount of alkylating metabolites present over a longer time duration, and lengthened the antileukemic effect of cyclophosphamide. Similar results were observed by Oira et al (15), who found that the active metabolites of cyclophosphamide were increased above controls in rats pretreated with phenobarbital and were decreased in rats that received chloramphenicol prior to treatment with cyclophosphamide. The effects of phenobarbital and pregnenolone-16α-carbonitrile (PCN), an agent also thought to induce drug metabolizing enzymes, on cyclophosphamide were studied in Walker 256 tumors implanted in rats (16). Phenobarbital pretreatment appeared to increase cyclophosphamide toxicity as measured by loss of body weight and early death, although tumor regression was observed. PCN pretreatment prevented mortality and body weight loss, but did not abolish the antineoplastic action of cyclophosphamide. Sladek (17) noted that pretreatment with phenobarbital increased the rate of metabolism of cyclophospha-

TABLE 1.—Compounds altering the plasma levels of cyclophosphamide

Increased plasma levels	Decreased plasma levels
Phenobarbital*	Phenobarbital*
Prednisone*	SKF-525A
Triiodothyronine	Chloramphenicol
Chlorpromazine	Apomorphine
Chloroquine	Corticosteroids*
	Nicotine
	Atropine
	Ephedrine
	Cocaine
	Dapsone

*Experimental evidence is available which indicates that phenobarbital and steroids may either increase or decrease the plasma levels of cyclophosphamide. The details of these experiments are reviewed in the text.

mide and that pretreatment with 3-methylcholanthrene, thioacetamide, morphine, or cobalt chloride depressed cyclophosphamide metabolism in rats bearing Walker 256 carcinosarcoma. Alberts and van Daalen Wetters (18,19) have recently shown that phenobarbital pretreatment markedly reduced the antileukemic activity of cyclophosphamide by reducing the total plasma alkylating metabolites, while allopurinol enhanced the cyclophosphamide effect using the spleen colony assay system and survival studies in mice bearing P388 leukemia. Although data for the phenobarbital-cyclophosphamide interaction in animal systems are confusing, phenobarbital probably reduces total cyclophosphamide alkylating metabolites although "peak" plasma levels for the active metabolites may actually be increased. Despite numerous animal studies which suggest that liver microsomal inducing agents alter cyclophosphamide activity, similar data in man are lacking. Bagley et al (20) reviewed the clinical pharmacology of radiolabeled cyclophosphamide in 26 patients. Although individual variation in plasma cyclophosphamide half-life and peak alkylating levels were noted following prior exposure to pentobarbital, dilantin, or prednisone, the authors noted no significant difference in the total (concentration × time) product for a given cyclophosphamide dose. In contrast to most animal studies, they suggest that alterations in the rate of cyclophosphamide metabolism by other agents do not alter the toxicity or therapeutic effect.

The effect of prednisolone on the activation of cyclophosphamide was studied by Hayakawa and co-workers (21). They pretreated rat liver slices with prednisolone and observed inhibition of cyclophosphamide activation as measured by a reduction in serum alkylating activity. Similar results were obtained in vivo when serum concentrations of cyclophosphamide were determined after ip administration in rats. Simultaneous administration of prednisolone inhibited activation of cyclophosphamide and decreased the ratio of active/inactive metabolites in serum samples. Hayakawa et al suggest that the prednisolone inhibition involves $NADPH_2$-dependent enzyme systems in the liver, perhaps by a competitive mechanism whereby prednisolone hydroxylation and cyclophosphamide activation compete for these enzyme systems. However, Hanasono and Fischer (22) found no effect of corticosteroids on the plasma alkylating activity of cyclophosphamide. It has been suggested that the dose of cyclophosphamide be reduced when simultaneously administered corticosteroids are discontinued (23). This suggestion has been criticized by others (24) who found that single doses of prednisone in man have an immediate inhibitory effect on cyclophosphamide activation, while longer term daily treatment with prednisone produced an increase in the rate of cyclophosphamide activation. They suggest that cyclophosphamide toxicity would be decreased upon withdrawal of prednisone. Kovacs and Steinberg (25) investigated the effects of chloroquine and prednisolone on cyclophosphamide toxicity in mice and found that chloroquine increased cyclophosphamide toxicity as measured by cumulative mortality, median survival, and peripheral leukocyte counts, whether single large doses (300–600 mg/kg) or daily smaller doses (40 mg/kg) of cyclophosphamide were given. High-dose prednisolone therapy (20 mg/kg/day × 8 days), but not low-dose (5 mg/kg/day × 8 days), also increased cyclophosphamide toxicity. The diversity of these data suggests that both the dose and timing of corticosteroid administration, as well as different animal species or strains, may affect cyclophosphamide toxicity. However, their combined use in a number of combination chemotherapy protocols has not been precluded by conflicting data on their interactions.

When chloramphenicol, an antibiotic which inhibits microsomal enzymes, was used to pretreat rats prior to cyclophosphamide therapy, the in vivo conversion of cyclophosphamide to the active alkylating metabolites was decreased. Chloramphenicol also increased both the mean day of death and the mean lethal dose of cyclophosphamide (26). The effects of a number of compounds on cyclophosphamide activity and metabolism were studied in L1210 leukemic mice and in human epidermoid carcinoma No. 2 cells (27). Nicotine, atropine, ephedrine, apomorphine, and cocaine were potent inhibitors of the microsomal enzyme conversion of cyclophosphamide to its active metabolite, aldophosphamide, while cytochrome C, SKF-525A, and some steroid hormones were less potent inhibitors. Triiodothyronine or chlorpromazine, compounds capable of impairing formation of energy-rich phosphate compounds used in oxidative metabolism, were given to rats bearing transplantable tumors receiving cyclophosphamide therapy (28). The combined administration of cyclophosphamide and either one of these drugs inhibited tumor growth more than cyclophosphamide alone, a result attributed to uncoupling of oxidative phosphorylation in the tumor cell. In an interesting report of a drug interaction for which no mechanism is clearly implicated, four of eight patients receiving dapsone together with cyclophosphamide for the treatment of leprosy experienced inhibition of the leukopenic effect of cyclophosphamide when compared to patients receiving smaller doses of cyclophosphamide alone (29). Finally, Hartshorn (7) reviewed a German study in which two patients

receiving insulin, carbutamide, and cyclophosphamide developed remarkable hypoglycemia, an effect attributed to cyclophosphamide-induced release of insulin from insulin-bearing antibodies.

These studies demonstrate the interactions of drugs often used clinically with cyclophosphamide on the metabolism of this agent. The relevance of these experimental studies to the use of this agent in man is uncertain, but an awareness of potential interactions of cyclophosphamide with other drugs, especially agents capable of inducing liver microsomal enzyme systems, may facilitate the clinician's perception of altered drug activity. The remaining alkylating agents do not require activation in man and are, therefore, not likely to be subject to the same interactions described for cyclophosphamide. However, alkylating agents have been shown to inhibit hepatic microsomal enzymes and may, therefore, affect the activation or degradation of other drugs by this mechanism (30). Specific adverse drug interactions for agents other than cyclophosphamide have not been reported.

ANTIMETABOLITES

Folic Acid Antagonists

Methotrexate is the most widely used antifolate. It exerts its cytocidal effect by inhibiting the enzyme, dihydrofolate reductase, and thereby inhibiting DNA, RNA, and protein synthesis within the cell (table 2). The action of methotrexate can be reversed by the administration of citrovorum factor (folinic acid), a derivative of tetrahydrofolic acid, which bypasses the block of dihydrofolate reductase induced by methotrexate (31). This effect is not a drug interaction in the usual sense, but rather the substitution of a necessary metabolite at a step beyond the methotrexate block of folic acid metabolism. Therapeutic implications of this reaction and experience in clinical trials have recently been reviewed (32).

Methotrexate is well absorbed following oral administration and approximately 80% of the drug is excreted in the urine within 24 hours after administration of conventional doses. Several investigators have attempted to alter methotrexate efficacy and toxicity by administering other drugs which interfere with its absorption or renal excretion. Zaharko et al (33) investigated the effects of concurrently administered antibiotics on methotrexate absorption in mice. Neomycin and sulfathiazole destroyed bacterial flora involved in gastrointestinal metabolism of methotrexate but absorption of methotrexate was inhibited by these antibiotics, as measured by increased fecal excretion of radiolabeled methotrexate. However, since metabolism of the drug in man is minimal, the clinical effect of concomitant antibiotic administration is unclear. Impairment of glomerular filtration is likely to increase the toxicity and antitumor effect of a drug whose major route of excretion is urinary by in-

TABLE 2.—Compounds altering the activity of methotrexate

Drug	Effect on methotrexate	Mechanism
Citrovorum factor	Reversal	Bypasses enzyme block
Sulfathiazole	Decreases absorption	Destroys gut bacteria
Neomycin	Decreases absorption	Destroys gut bacteria
Salicylates	Alters plasma level	Decreases renal clearance; displaces methotrexate from plasma proteins
Sulfonamides	Alters plasma level	Displaces methotrexate from plasma proteins
Barbiturates	Alters plasma level	Displaces methotrexate from plasma proteins
Tranquilizers	Alters plasma level	Displaces methotrexate from plasma proteins
Diphenylhydantoin	Alters plasma level	Displaces methotrexate from plasma proteins
Corticosteroids	Decreases intracellular level	Inhibits methotrexate cellular uptake
Penicillin	Decreases intracellular level	Inhibits methotrexate cellular uptake
Kanamycin	Decreases intracellular level	Inhibits methotrexate cellular uptake
Triamterene	"Pseudo-resistance"	Increases dihydrofolate reductase level
Allopurinol	Decreases antitumor effect	Increases intracellular purine level

creasing the time of exposure to normal and neoplastic tissues. Other medications have been shown to alter the renal clearance of methotrexate in patients with leukemia and other malignancies (14). Fifteen patients with acute leukemia or disseminated malignancies, 14 of whom had normal renal function, were given methotrexate and the renal clearances of para-aminohippurate (PAH) and inulin were measured. The clearance of methotrexate exceeded the inulin clearance in all cases, suggesting active renal tubular secretion of the drug. When weak organic acids, notably salicylate, and high concentrations of PAH were given, the methotrexate clearance fell below the glomerular filtration rate. Sulfisoxazole did not alter methotrexate clearance to a significant degree. However, since many of the commonly used antibiotics are salts of weak organic acids, other adverse interactions might be expected to occur. An additional observation made by these investigators was that salicylate and sulfisoxazole displaced methotrexate from plasma protein binding, an effect not well correlated with changes in renal clearance. Since the binding of a drug to serum albumin lowers the amount of free drug available at sites of action in direct proportion to the fraction of drug bound to albumin (10), an altered therapeutic effect as well as altered toxicity might be expected to occur. Plasma protein binding was also altered by para-aminobenzoic acid, sulfonamides, and salicylates according to another report (11). These drugs increased the concentration of free methotrexate in vitro when present in concentrations 50–500 times that of methotrexate. Barbiturates, tranquilizers, and diphenylhydantoin are also reported to increase methotrexate toxicity by displacement from binding to plasma proteins (34,35). Agents which displace methotrexate from plasma proteins increase the availability of methotrexate to tissue sites and might be expected to augment toxicity. As such, the concomitant administration of such drugs with methotrexate, when necessary, requires close clinical monitoring.

Studies by Bender (36) and Goldman et al (37,38) describe a carrier-mediated transport process for the transmembrane movement of methotrexate and naturally occurring folates in L1210 murine leukemia and in human leukemia cells. Uptake of methotrexate may be reduced by other folates, especially folic acid and folinic acid, by organic anions such as antibiotics and hydrocortisone, and by a variety of other drugs. Zager et al (39) investigated the effects of several clinically useful antibiotics and antineoplastic agents on the intracellular accumulation of methotrexate and on its antitumor effects in L1210 leukemia-bearing mice. Using doses felt to be estimates of achievable blood levels in man after usual single iv doses, corticosteroids and cephalothin were found to have the greatest effect on methotrexate uptake with lesser effects noted for penicillin and kanamycin. Hydrocortisone at 10^{-5} and 10^{-3} M showed the greatest inhibition of methotrexate uptake and antagonized the antitumor effect of methotrexate in vivo, as measured by mean animal survival time, when hydrocortisone preceded methotrexate administration. Studies with human leukemia cells confirmed the inhibitory effects of cephalothin and hydrocortisone on methotrexate uptake (40). Finally, triamterene has been found to elevate levels of dihydrofolate reductase in human leukocytes when given as a diuretic (41). As such, "pseudo-resistance" could be induced requiring increased amounts of methotrexate to achieve a therapeutic effect.

Finally, the effects of allopurinol on the antitumor effects of methotrexate in L1210 and P388 murine leukemias were investigated (42). The concurrent administration of allopurinol partially reversed the antitumor effects of methotrexate against L1210 leukemia when mean animal survival was measured, although efficacy against P388 leukemia was not altered. Methotrexate growth-inhibitory effects on cells in tissue culture were not affected by allopurinol, nor was methotrexate toxicity altered by allopurinol as measured by LD50 studies. Although further studies are required to better define this interaction, an accumulation of purines within the cell due to decreased catabolism as a result of xanthine oxidase inhibition by allopurinol may abort the "purineless death" of tumor cells induced by methotrexate (43).

Purine Analogs

The two purine antagonists of major clinical importance are 6-mercaptopurine and 6-thioguanine. Both are rapidly absorbed from the gastrointestinal tract following oral administration, have plasma half-lives of about 90 minutes, and are excreted mainly in the urine as metabolites of the parent compound. While these metabolic pathways are known to be perturbed by mesantoin and phenobarbital (44), their specific effect on antitumor activity remains to be defined.

Most of the reported drug interactions with purine antagonists involve allopurinol. Allopurinol is a structural analog of hypoxanthine and acts by competing with other purines for the catabolic site of xanthine oxidase, thus preventing oxidation of purines to uric acid. Because xanthine oxidase is required for the metabolism of hypoxanthine to xanthine and of xanthine to uric acid, allopurinol has clinical utility in reducing uric acid formation

and subsequent hyperuricemia. 6-Mercaptopurine undergoes a xanthine oxidase catalyzed conversion to thiouric acid during detoxification; therefore, concurrent therapy with allopurinol will potentiate the effects of 6-mercaptopurine, increase plasma levels while delaying excretion, and thus increase toxicity. A reduction of the 6-mercaptopurine dose to 25%–30% of the usual dose is recommended when allopurinol is also given. The dosage of 6-thioguanine does not need reduction, however, since its degradative pathway does not require xanthine oxidase (45). Elion and co-workers (46) investigated the effect of allopurinol on the metabolism of 6-mercaptopurine in mice bearing Adenocarcinoma 755 and in a patient who had received 6-mercaptopurine as therapy for chronic granulocytic leukemia. Urine was collected following 6-mercaptopurine administration with and without concurrent allopurinol and the amounts of 6-mercaptopurine and its metabolite, 6-thiouric acid, were determined. In both mouse and man, administration of allopurinol resulted in decreased metabolism of 6-mercaptopurine to 6-thiouric acid. When the antitumor effects of several substituted purines on mice bearing Adenocarcinoma 755 were measured, simultaneous allopurinol administration potentiated these effects while lowering the minimal effective dose without increasing toxicity. When daily oral administration of 300 mg of allopurinol accompanied therapy with 6-mercaptopurine with or without other antineoplastic agents, patients so treated showed a marked inhibition of the production of 6-thiouric acid (47). These patients had a variety of malignancies and were receiving a number of other medications in addition to the antineoplastic agents. When distribution and elimination half-lives and volume of distribution of 6-mercaptopurine were calculated, allopurinol did not seem to alter these values. When the plasma half-lives were measured in three patients receiving iv 6-mercaptopurine, no alteration was noted when allopurinol was simultaneously administered and withdrawn (47). It was concluded that a reduction of 6-mercaptopurine dosage is appropriate when allopurinol is also administered, but further investigation of this interaction was recommended. The clinician should be aware of this interaction and consider dose modification when 6-mercaptopurine and allopurinol are concurrently administered. This interaction was also cited as a possible cause for the development of severe pancytopenia and subsequent fatal sepsis in a recent clinicopathologic conference (48).

Pyrimidine Analogs

The fluorinated pyrimidines and their nucleosides are an important subgroup of this class of drugs with the major compound in clinical use being 5-fluorouracil. This drug has the same structure as the nucleotide uracil, except for a fluorine atom in position 5 of the ring. Another compound with antitumor activity is a nucleoside of 5-fluorouracil, 5-fluorodeoxyuridine. In vivo, 5-fluorouracil is converted to 5-fluoro-2′-deoxyuridine-5′-phosphate intracellularly, the metabolically active form of the agent. This metabolite acts by competing with deoxyuridylate for the enzyme, thymidylate synthetase, thereby inhibiting formation of thymidylic acid and subsequent production of DNA (1). The liver is the major site for catabolism of 5-fluorouracil and related compounds.

Surprisingly little is known about the adverse drug interactions of this class of antineoplastic agents. Those interactions which have been described have evolved from attempts to affect the metabolism and biologic activity of 5-fluorouracil by simultaneously administering other agents. Interactions with prednisolone were investigated as a result of the frequent inclusion of corticosteroids into combination chemotherapy protocols while phenobarbital was chosen for study because of its induction of liver microsomal enzymes (49). No evidence for an effect of prednisolone or phenobarbital pretreatment was observed on 5-fluorouracil metabolite excretion, an expected observation since 5-fluorouracil metabolism is thought to be due to soluble rather than microsomal liver enzymes. When corticosteroid and vincristine effects on 5-fluorouracil activity in L1210 cells were investigated, neither of these agents altered the ability of 5-fluorouracil to suppress de novo DNA synthesis as measured by deoxyuridine incorporation (50). Similar results were obtained using human bone marrow cells.

VINCA ALKALOIDS

Vinblastine and vincristine, two naturally occurring alkaloids extracted from *Vinca rosea*, the periwinkle plant, are in clinical use as antineoplastic agents. They are structurally alike except for replacement of the formyl group in vincristine with a methyl group in vinblastine. Their mechanism of action is not well understood but they appear to bind to the microtubular proteins of the mitotic spindle to produce arrest of dividing cells in metaphase. Pharmacologic studies in rats suggest rapid clearing of these drugs from the blood, followed by hepatic concentration and excretion into the biliary system and intestines with small amounts excreted in the urine. Sartorelli and Creasey (51) suggest that since binding of vinca alkaloids to microtubular

proteins occurs, they may also bind to other microtubular elements in neuronal processes, perhaps accounting for the neurotoxicity encountered with these compounds. Subsequent administration of other drugs may augment vincristine-induced neurotoxicity (52). Hildebrand and Kevis (52) describe three patients who had received vincristine in total doses of 11–18 mg given over 1–3 months with resultant paresthesias and absence of deep tendon reflexes. Isoniazid administration in two patients and L-asparaginase treatment in the third resulted in marked worsening of the paresthesias, accompanied by muscle weakness and eventual neurogenic atrophy. The relatively small doses of isoniazid administered, accompanied by pyridoxine, and the low doses of L-asparaginase were not felt to be sufficient, by themselves, to account for the increased neurotoxicity. Hildebrand and Kevis speculate that other potentially neurotoxic agents may augment the toxicity of vincristine.

The interaction of vincristine and vinblastine with serum and plasma proteins was recently studied by Donigan and Owellen (53). When the concentration of serum proteins was held constant, the amount of alkaloid bound was in direct proportion to the concentration of free vinblastine, while vincristine displayed this relationship only at low and high concentrations of the alkaloid. No difference was found between plasma and serum binding for vincristine and vinblastine. When preparations of albumin and α, β, and γ globulins were analyzed for their ability to adsorb alkaloids, vincristine and vinblastine were both adsorbed more to the α- and β-globulin fractions. However, additional studies are required to define the role of plasma protein binding in altering the therapeutic effect.

STEROID HORMONES

Adrenocortical and sex hormones are used in the treatment of a variety of neoplasms. Most reported interactions between steroid hormones and other drugs involve the effects of these drugs on the metabolism of glucocorticoids (table 3). The effect of diphenylhydantoin on the pharmacokinetics of corticosteroids was studied in patients before and after therapy with diphenylhydantoin (54,55). Tritium-labeled cortisol was infused and its metabolic fate was determined. A decrease in disappearance time and an increase in the metabolic clearance rate were noted following diphenylhydantoin administration (54). Choi et al (54) suggest that diphenylhydantoin enhances tissue uptake of cortisol primarily at the hepatic smooth endoplasmic reticulum since an increase in hydroxylated cortisol formation was observed. Similar results were obtained when radiolabeled dexamethasone was injected and blood and urine were collected before and after 3–4 weeks of diphenylhydantoin treatment in usual therapeutic doses (55). A decrease in plasma half-life and an increase in metabolic clearance rate following diphenylhydantoin administration were again observed. Other investigators reached a similar conclusion when studies were performed in rats. Although gastrointestinal absorption of dexamethasone was not impaired in rats treated with diphenylhydantoin, biliary excretion and hepatic conjugation of dexamethasone were enhanced (56). Changes in steroid metabolism by other drugs have been reviewed by Conney et al (57). The drugs investigated include those that stimulate drug-metabolizing activity in liver microsomal enzymes and those that stimulate steroid hydroxylase activity in the liver. The administration of compounds known to stimulate microsomal drug metabolism also stimulated hydroxylation of steroid hormones in test animals. The drugs studied included phenobarbital, chlorcyclizine, and phenylbutazone. Similar metabolic effects were also noted when the uterotropic action of 17β-estradiol and estrone on rats pretreated with any of these agents was examined (58). The clinical relevance of the effect of drugs altering hepatic microsomal activity and steroid hydroxylation on corticosteroid activity is unknown.

Additional interactions of corticosteroids with other drugs include reduction in the renal excretion of conjugated corticosteroids in normal subjects following oral administration of 0.6 g of aspirin (59), a report of steroid displacement from plasma proteins by phenylbutazone and indomethacin (60), and a reduction in dicumarol requirements for anticoagulation following myocardial infarction in patients also treated with prednisone or cortisone acetate (61,62).

MISCELLANEOUS AGENTS

Procarbazine hydrochloride, an *N*-methylhydrazine derivative, is an antitumor agent that is well absorbed orally and primarily excreted in the urine. Its mechanism of antitumor action appears to involve alteration of nucleic acid synthesis, probably by fragmentation of DNA. In addition, procarbazine is a potent inhibitor of monoamine oxidase (63). An interaction between procarbazine and alcohol has been described with a syndrome including headache, diaphoresis, and erythematous facies following alcohol consumption (64,65). This interaction may be due to procarbazine effects on alcohol detoxifying enzymes. Procarbazine has also been found to deplete plasma pyridoxal phosphate (66), an action similar to another hydrazine derivative,

TABLE 3.—Compounds altering the activity of corticosteroids

Drug	Effect on steroids	Mechanism
Diphenylhydantoin	Decreases activity	Increases liver enzyme metabolism
Phenobarbital	Decreases activity	Increases liver enzyme metabolism
Phenylbutazone	Decreases activity	Increases liver enzyme metabolism
Chlorcyclizine	Decreases activity	Increases liver enzyme metabolism
Salicylates	Increases plasma level	Decreases renal clearance
Phenylbutazone	Increases plasma level	Displaces steroid from plasma proteins
Indomethacin	Increases plasma level	Displaces steroid from plasma proteins

isoniazid, whose clinical neurotoxicity has been successfully reversed by simultaneous administration of pyridoxine. Attempts to reverse procarbazine toxicity by administering pyridoxine have been unsuccessful (63,67) and have actually potentiated procarbazine-induced convulsions in mice (66). Phenothiazine derivatives, barbiturates, tricyclic antidepressants, and narcotics are reportedly potentiated by procarbazine (64,68) and the concurrent use of these agents should be attempted very cautiously (69).

Adriamycin and daunorubicin are relatively new antineoplastic agents and are members of a class of compounds known as anthracycline antibiotics derived from the fungus *Streptomyces peucetius* var. *caesius*. Their exact mechanism of antitumor action is not known, but may involve binding to base pairs on the DNA strand with subsequent inhibition of DNA-directed RNA polymerase. Adriamycin metabolism is influenced by liver function and an enhanced rate of disappearance of this drug was observed in mice pretreated with phenobarbital (70). The mechanism for this interaction is liver microsomal enzyme induction and Reich and Bachur (70) recommend avoiding the concurrent use of drugs known to induce liver microsomal enzymes for optimal adriamycin effect. One of the major dose-limiting toxicities of adriamycin is cardiotoxicity. Adriamycin and the digitalis glycosides have structural similarities and possible competition between these classes of agents as a means of decreasing anthracycline cardiotoxicity has been investigated. Strophanthin G, a cardiac glycoside, reversed the adriamycin-induced decline in myocardial contractility and the decline in coronary blood flow in dog studies (71); however, strophanthin G pretreatment did not decrease adriamycin accumulation by the heart in mice receiving single or multiple doses of these agents (72). Recent work by Myers et al (73) suggests that pretreating healthy mice with α-tocopherol (vitamin E) diminishes the cardiotoxicity of adriamycin when compared with untreated controls. However, the clinical role of pretreatment with cardiac glycosides or with vitamin E in an attempt to reduce anthracycline cardiotoxicity remains to be determined.

The class of antineoplastic agents known as nitrosoureas has activity against a variety of neoplasms. Their lipid solubility allows access to the cerebrospinal fluid and activity of these agents against central nervous system neoplasms has been shown. The exact mechanism of their antitumor activity is not known, but may involve alkylation with cross linking of DNA strands. Reported interactions of nitrosoureas with other agents have developed from attempts to enhance their antitumor effect by concurrently administering other drugs. Amphotericin B, a polyene antifungal agent, combined with BCNU inhibited RNA synthesis and affected cell kill in mouse L cells, although neither agent alone at the same concentrations had any effect (74). Further, enhanced survival with this combination in AKR leukemic mice was also shown. Medoff et al (74) attributed the effect to a nonspecific enhancement of cellular immunity, perhaps implying selectivity of polyenes for tumor cells. Although they did not find enhancement of BCNU activity by another polyene, vitamin A, other investigators (75) observed an enhanced antitumor effect of BCNU in murine L1210 leukemia when vitamin A alcohol, at a dose tolerable in man, was given 3–6 hours before the BCNU. When caffeine was added to the vitamin A-BCNU combination, further enhancement of survival in L1210 leukemic mice was noted (76). However, the relevance of these observations to clinical trials remains speculative at this time.

Finally, allopurinol, an agent often used in conjunction with antineoplastic therapy to minimize uric acid toxicity, has been reported to cause an

unusual hypersensitivity reaction (77). Two patients receiving allopurinol experienced severe vasculitis accompanied by renal failure and both had received prior therapy with thiazide diuretics, among other drugs. The exact mechanism for this interaction remains to be determined (table 4).

CONCLUSIONS

Considering the incidence of cancer in the world today and the relative frequency with which antineoplastic agents are used, relatively little is known of their drug interactions, potential or documented. It is hoped that this paper will provide an impetus for further study of these interactions and for increased scrutiny of patients receiving therapy.

REFERENCES

1. CHABNER BA, MYERS CE, COLEMAN CN, ET AL. The clinical pharmacology of antineoplastic agents. (Parts 1 and 2.) N Engl J Med 292:1107–1113, 1159–1168, 1975.
2. MARTIN EW, ALEXANDER SF, HASSAN WE, ET AL. Hazards of medication: a manual on drug interactions, incompatibilities, contraindications, and adverse effects. Philadelphia, J. B. Lippincott Co, 1971, pp 1–16.
3. SMITH JW, SEIDL LG, and CLUFF LE. Studies on the epidemiology of adverse drug reactions. V. Clinical factors influencing susceptibility. Ann Intern Med 65:629–640, 1966.
4. MORRELLI HF, and MELMON KL. The clinician's approach to drug interactions. Calif Med 109:380–389, 1968.
5. APPLE MA. Avoiding interactions in anticancer drug therapy. Drug Ther, 1975, pp 203–214.
6. CLARK TH, CONNEY AH, HARPOLE BP, ET AL. Drug interactions that can affect your patients. Patient Care 1:33–71, 1967.
7. HARTSHORN EA. Drug interactions: antineoplastics. Drug Intell 3:174–180, 196–197, 1969.
8. COHEN SN, and ARMSTRONG MF. Drug Interactions: A Handbook for Clinical Use. Baltimore, Williams and Wilkins Co, 1974, pp xiii–xviii.
9. PRESCOTT LF. Pharmacokinetic drug interactions. Lancet 2:1239–1243, 1969.
10. KOCH-WESER J, and SELLERS EM. Drug therapy: binding of drugs to serum albumin. N Engl J Med 294:311–316, 526–531, 1976.
11. DIXON RL, HENDERSON ES, and RALL DP. Plasma protein binding of methotrexate and its displacement by various drugs. Fed Proc 24:454, 1965.
12. FIELD RB, GANG M, KLINE I, ET AL. The effect of phenobarbital or 2-diethylaminoethyl-2,2-diphenylvalerate on the activation of cyclophosphamide in vivo. J Pharmacol Exp Ther 180:475–483, 1972.
13. ELION GB, CALLAHAN S, RUNDLES RW, ET AL. Relationship between metabolic fates and antitumor activities of thiopurines. Cancer Res 23:1207–1217, 1963.
14. LIEGLER DG, HENDERSON ES, HAHN MA, ET AL. The effect of organic acids on renal clearance of methotrexate in man. Clin Pharmacol Ther 10:849–857, 1969.
15. OIRA S, MAAZAWA S, IRINODA Y, ET AL. Increased antitumor activity of cyclophosphamide following pretreatment with inducers of drug-metabolizing enzymes (cytochrome P-450). Tohoku J Exp Med 114:55–60, 1974.
16. KOVACS K, KHANDEKAS JD, TUCHWEBER B, ET AL. Effect of phenobarbital and pregnenolone-16α-carbonitrile on the toxic and antineoplastic action of cyclophosphamide. Naturwissenschaften 58:526, 1971.
17. SLADEK NE. Therapeutic efficacy of cyclophosphamide as a function of its metabolism. Cancer Res 32:535–542, 1972.
18. ALBERTS DS, and VAN DAALEN WETTERS T. The effect of phenobarbital on cyclophosphamide antitumor activity. Cancer Res 36:2785–2789, 1976.

TABLE 4.—Compounds altering the effect of other antineoplastic agents

Antineoplastic	Drug	Effect on antineoplastic	Mechanism
6-Mercaptopurine	Allopurinol	Increases plasma level	Blocks enzyme metabolism
Procarbazine	Alcohol	Disulfarim-like effect	Procarbazine inhibits monoamine oxidase
	Phenothiazine	Non-antineoplastic activity potentiated	Procarbazine inhibits monoamine oxidase
	Barbiturates	Non-antineoplastic activity potentiated	Procarbazine inhibits monoamine oxidase
	Antidepressants	Non-antineoplastic activity potentiated	Procarbazine inhibits monoamine oxidase
	Narcotics	Non-antineoplastic activity potentiated	Procarbazine inhibits monoamine oxidase
Adriamycin	Phenobarbital	Reduces antitumor effect	Increases clearance from plasma
BCNU	Amphotericin B	Enhances antitumor effect	Possibly enhances cellular immunity
	Vitamin A alcohol	Enhances antitumor effect	Possibly enhances cellular immunity
Allopurinol	Thiazide diuretics	Hypersensitivity reaction	Unknown

19. ———. The effect of allopurinol on cyclophosphamide antitumor activity. Cancer Res 36:2790–2794, 1976.
20. BAGLEY CM, BOSTICK FW, and DEVITA VT. Clinical pharmacology of cyclophosphamide. Cancer Res 33:226–233, 1973.
21. HAYAKAWA T, KANAI N, YAMADA R, ET AL. Effect of steroid hormone on activation of Endoxan (cyclophosphamide). Biochem Pharmacol 18:129–135, 1969.
22. HANASONO GK, and FISCHER LJ. Plasma levels and urinary excretion of [^{14}C] cyclophosphamide and its radioactive metabolites in rats pretreated with prednisolone. Biochem Pharmacol 21:272–276, 1972.
23. KAPLAN SR, and CALABRESI C. Immunosuppressive agents. N Engl J Med 289:952–955, 1973.
24. FABER OK, and MOURIDSEN HT. Cyclophosphamide activation and corticosteroids. N Engl J Med 291:211, 1974.
25. KOVACS K, and STEINBERG AD. Cyclophosphamide drug interactions and bone marrow transplantation. Transplantation 13:316–321, 1972.
26. DIXON RL. Effect of chloramphenicol on the metabolism and lethality of cyclophosphamide in rats. Proc Soc Exp Biol Med 127:1151–1155, 1968.
27. HILL DL, LASTER WR, and STRUCK RF. Enzymatic metabolism of cyclophosphamide and nicotine and production of a toxic cyclophosphamide metabolite. Cancer Res 32:658–665, 1972.
28. BACIGALUPO G. Uncoupling the oxidative phosphorylation in the combined chemotherapy of cancer. Acta Unio Int Cancr 20:52–53, 1964.
29. DAVISON AR, SCHULZ EJ, FALKSON G, ET AL. Effect of cyclophosphamide on leprosy: competitive action of cyclophosphamide and dapsone. Cancer 2:1138–1142, 1964.
30. TARDIFF RG, and DUBOIS KP. Inhibition of hepatic microsomal enzymes by alkylating agents. Arch Int Pharmacodyn Ther 177:445–456, 1969.
31. GOLDIN A, VENDITTI JM, KLINE I, ET AL. Eradication of leukemic cells (L1210) by methotrexate plus citrovorum factor. Nature 212:1548–1550, 1966.
32. BENDER RA. Antifolate resistance in leukemia: treatment with "high-dose" methotrexate and citrovorum factor. Cancer Treat Rev 2:215–224, 1975.
33. ZAHARKO DS, BRUCKNER H, and OLIVERIO VT. Antibiotics alter methotrexate metabolism and excretion. Science 166:887–888, 1969.
34. HANSTEN PD. Drug Interactions. Philadelphia, Lea and Febiger, 1971, pp 145–146.
35. REILLY MJ. Drug information digest: methotrexate U.S.P. (amethopterin) and methotrexate sodium. Am J Hosp Pharm 30:542–548, 1973.
36. BENDER RA. Membrane transport of methotrexate (NSC-740) in human neoplastic cells. Cancer Chemother Rep Part 3, vol 6(1):73–82, 1975.
37. GOLDMAN ID. The characteristics of the membrane transport of amethopterin and the naturally occurring folates. Ann NY Acad Sci 186:400–422, 1971.
38. GOLDMAN ID, LICHTENSTEIN NS, and OLIVERIO VT. Carrier-mediated transport of the folic acid analogue, methotrexate, in the L1210 leukemia cell. J Biol Chem 243:5007–5017, 1968.
39. ZAGER RF, FRISBY SA, and OLIVERIO VT. The effects of antibiotics and cancer chemotherapeutic agents on the cellular transport and antitumor activity of methotrexate in L1210 murine leukemia. Cancer Res 33:1670–1675, 1973.
40. BENDER RA, BLEYER WA, FRISBY SA, ET AL. Alteration of methotrexate uptake in human leukemia cells by other agents. Cancer Res 35:1305–1308, 1975.
41. ROBERTS DW, and HALL TC. Triamterene-induced elevation of dihydrofolate reductase activity in human leukocytes. Biochem Pharmacol 17:484–487, 1968.
42. GRINDEY G, and MORAN R. Effects of allopurinol on the therapeutic efficacy of methotrexate. Cancer Res 35:1702–1705, 1975.
43. HRYNIUK WM. Purineless death as a link between growth rate and cytotoxicity by methotrexate. Cancer Res 32:1506–1511, 1972.
44. GOLDIN A, VENDITTI JM, and KLINE I. Evaluation of antileukemic agents employing advanced leukemia L1210 in mice. II. Cancer Res 20:382–388, 1960.
45. SALMON SE, and APPLE M. Chemotherapeutic agents: cancer chemotherapy. Rev Med Pharmacol 4:477–479, 1974.
46. ELION GB, CALLAHAN S, NATHAN H, ET AL. Potentiation by inhibition of drug degradation: 6-substituted purines and xanthine oxidase. Biochem Pharmacol 12:85–93, 1963.
47. COFFEY JJ, WHITE CA, LESK AB, ET AL. Effect of allopurinol on the pharmacokinetics of 6-mercaptopurine in cancer patients. Cancer Res 32:1283–1289, 1972.
48. NIES AS, and OATES JA. Clinicopathologic conference: hypertension and the lupus syndrome revisited. Am J Med 51:812–814, 1971.
49. AMBRE JJ, and FISCHER LJ. The effect of prednisolone and other factors on the catabolism of 5-fluorouracil in rats. J Lab Clin Med 78:343–353, 1971.
50. BRUCKNER HW, SCHREIBER C, and WAXMAN S. Interaction of chemotherapeutic agents with methotrexate and 5-fluorouracil and its effect on de novo DNA synthesis. Cancer Res 35:801–806, 1975.
51. SARTORELLI AC, and CREASEY WA. Cancer chemotherapy. Annu Rev Pharmacol 9:51–72, 1969.
52. HILDEBRAND J, and KEVIS Y. Additive toxicity of vincristine and other drugs for the peripheral nervous system. Acta Neurol Belg 71:486–491, 1971.
53. DONIGAN DW, and OWELLEN RJ. Interaction of vinblastine, vincristine and colchicine with serum proteins. Biochem Pharmacol 22:2113–2119, 1973.
54. CHOI Y, THRASHER K, WERK EE, ET AL. Effect of diphenylhydantoin on cortisol kinetics in humans. J Pharmacol Exp Ther 176:27–34, 1971.
55. HAQUE N, THRASHER K, WERK EE, ET AL. Studies on dexamethasone metabolism in man: effect of diphenylhydantoin. J Clin Endocrinol Metab 34:44–50, 1972.
56. JULEIZ W, MEIKLE AW, LEVISON RA, ET AL. Effect of diphenylhydantoin on the metabolism of dexamethasone. N Engl J Med 283:11–14, 1970.
57. CONNEY AH, SCHNEIDMAN K, JACOBSON M, ET AL. Drug-induced changes in steroid metabolism. Ann NY Acad Sci 123:98–109, 1965.
58. LEVIN W, and WELCH RM. Effect of phenobarbital and other drugs on the metabolism and uterotropic action of estradiol 17-β and estrone. J Pharmacol Exp Ther 159:362–371, 1968.
59. ELLIOT HC. Reduced adrenocortical steroid excretion rates in man following aspirin administration. Metabolism 11:1015–1018, 1962.
60. MAICKEL RP, MILLER FP, and BRODIE BB. Interaction of non-steroidal anti-inflammatory agents with corticosteroids. Pharmacologist 7:182, 1965.
61. GERISCHE RA, JOHNSON AS, ANDERSON WL, ET AL. Acute myocardial infarction in man treated with cortisone. Harper Hosp Bull 19:197–200, 1961.
62. SIEVERS J, JOHANSSON BW, and NILSSON SE. The corticosteroid treatment of acute myocardial infarction. Cardiologica 45:65–76, 1964.
63. DEVITA VT, HAHN MA, and OLIVERIO VT. Monoamine oxidase inhibition by a new carcinostatic agent, *n*-isopropyl-α-(2-methylhydrazino)-*p*-toluamide, (MIH). Proc Soc Exp Biol Med 120:561–565, 1965.
64. BRULE G, SCHLUMBERGER JR, and GRISCELLI C. *N*-isopropyl-α-(2-methylhydrazino)-*p*-toluamide, hydrochloride (NSC-77213)

in treatment of solid tumors. Cancer Chemother Rep 44:31–38, 1965.
65. MATHÉ G, SCHWEISGUTH O, SCHNEIDER M, ET AL. Methylhydrazine in treatment of Hodgkin's disease and various forms of haematosarcoma and leukaemia. Lancet 2:1077–1080, 1963.
66. CHABNER BA, DEVITA VT, CONSIDINE N, ET AL. Plasma pyridoxal phosphate depletion by the carcinostatic procarbazine. Proc Soc Exp Biol Med 132:1119–1122, 1969.
67. BRUNNER KW, and YOUNG CW. A methylhydrazine derivative in Hodgkin's disease and other malignant neoplasms: therapeutic and toxic effects studied in 51 patients. Ann Intern Med 63:69–86, 1965.
68. BILLMEIER GJ, and HOLTON CP. Procarbazine hydrochloride in childhood cancer. J Pediatr 75:892–895, 1969.
69. WEISS HD, WALKER MD, and WIERNIK PH. Neurotoxicity of commonly used antineoplastic agents. N Engl J Med 291:75–81, 127–133, 1974.
70. REICH SD, and BACHUR NR. Alterations in adriamycin efficacy of phenobarbital. Cancer Res 36:3803–3806, 1976.
71. ARENA E, D'ALESSANDRO N, DUSONCHET L, ET AL. Influence of pharmacokinetic variations on the pharmacologic properties of adriamycin. *In* International Symposium on Adriamycin (Carter SK, DiMarco A, Ghione M, et al, eds). Berlin, Springer-Verlag, 1972, pp 96–116.
72. BACHUR NR, RUTER W, and ARENA E. Cardiac uptake of adriamycin (NSC-123127) not affected by strophanthin G (NSC-25485). Cancer Chemother Rep 59:765–766, 1975.
73. MYERS CE, MCGUIRE W, and YOUNG R. Adriamycin: amelioration of toxicity by α-tocopherol. Cancer Treat Rep 60:961–962, 1976.
74. MEDOFF G, SCHLESSINGER D, and KOBAYASKI GS. Brief communication: Polyene potentiation of antitumor agents. J Natl Cancer Inst 50:1047–1050, 1973.
75. COHEN MH, and CARBONE PP. Enhancement of the antitumor effects of 1,3-bis(2-chloroethyl)-1-nitrosourea and cyclophosphamide by vitamin A. J Natl Cancer Inst 48:921–926, 1972.
76. COHEN MH. Enhancement of the antitumor effect of 1,3-bis(2-chloroethyl)-1-nitrosourea by vitamin A and caffeine. J Natl Cancer Inst 48:927–932, 1972.
77. YOUNG JL, BOSWELL RB, and NIES AS. Severe allopurinol hypersensitivity: association with thiazides and prior renal compromise. Arch Intern Med 134:533–558, 1974.

Epipodophyllotoxin VM-26 in the Treatment of Childhood Neuroblastoma [1,2,3]

Gaston Rivera,* Alexander Green, Ann Hayes, Thomas Avery, and Charles Pratt [4,5]

SUMMARY

Thirteen children with disseminated neuroblastoma that had become refractory to conventional chemotherapy were treated with the epipodophyllotoxin VM-26. Three patients developed partial responses (> 50% reductions in tumors and in the proportion of tumor cells in bone marrow). Acute nonhematologic toxicity after treatment was minimal. Hematologic toxicity was observed but could not be assessed accurately since most patients had abnormal hematopoiesis due to extensive tumor involvement of bone marrow. These results demonstrate that VM-26, as a single agent, can produce measurable tumor responses in children with neuroblastoma.

[Cancer Treat Rep 61:1243–1248, 1977]

Neuroblastoma is one of the most common malignant tumors in children. Although the results of therapy, including long-term survival, in children with disseminated disease have been generally discouraging, a complete remission rate of 63% was recently reported for patients treated with cyclophosphamide-adriamycin combination chemotherapy (1). Despite this demonstration of sensitivity to chemotherapy, cures in children > 1 year of age remain infrequent. The search for new active compounds must continue if effective maintenance regimens are to be developed and long-term remissions promoted. A major obstacle to improving the prognosis is the limited number of drugs with clearly demonstrated effectiveness as single agents (2,3).

A recently introduced epipodophyllotoxin, VM-26, has shown oncolytic activity against transplanted murine tumors (4,5) and advanced human neoplasms, especially malignant lymphoma, brain tumors, bladder carcinoma, oat cell carcinoma of the lung, and ovarian cancer (6–11). In addition, we have reported preliminary observations of 39 children with cancer who received either VM-26 or its analog VP-16-213, or both, after failing to respond to conventional chemotherapy (12). Twenty-nine patients had acute leukemia and ten had solid tumors. Although definite clinical responses were obtained in nine of the 29 patients with acute leukemia, the general effectiveness of these drugs against solid tumors, including neuroblastoma, was not conclusive, largely because of the heterogeneity of tumor types among these patients.

Reported here are the results of a clinical trial in which 13 children with disseminated neuroblastoma were treated with VM-26. We demonstrate measurable antitumor activity by VM-26 with few serious toxic effects.

PATIENTS AND METHODS

Thirteen children[6] with disseminated neuroblastoma were admitted to the study. The group included eight white girls and five white boys. Their median age was 3 years with a range of 2–9 years. The median time from diagnosis of neuroblastoma to treatment with VM-26 was 10 months with a range of 6–19 months.

[1]Received Jan 6, 1977; revised Apr 6, 1977; accepted Apr 20, 1977.

[2]Supported by Public Health Service grant CA-08480 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare; and by ALSAC.

[3]VM-26: NSC-122819; 4′-demethylepipodophyllotoxin, 9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside). Supplied by the Investigational Drug Branch, Division of Cancer Treatment, NCI, Bethesda, Md.

[4]St. Jude Children's Research Hospital, Memphis, Tenn.

[5]Editorial assistance was provided by John Gilbert and M. Jane Seifert.

**Reprint requests to*: Dr. G. Rivera, St. Jude Children's Research Hospital, PO Box 318, Memphis, Tenn 38101.

[6]3 patients were reported earlier (12).

TABLE 1.—Responses to VM-26

Patient No.	Race/sex/ age (yrs)*	Duration of disease (mos)	Measurable disease before treatment	Single dose (mg/m^2)	Total dose (mg/m^2)	Days on study	Response to therapy†
1	W/B/2	6	Bone marrow, tumors (skull, orbit, mandible), hepatomegaly, lymphadenopathy	50	175	13	NE
2	W/B/2	9	Bone marrow, hepatomegaly, tumors (mandible)	100	300	10	NE
3	W/G/7	10	Bone marrow, tumor (skull), multiple skeletal metastases	100	350	31	NE‡
4	W/G/3	10	Bone marrow, tumors (orbits), lymphadenopathy	100	700	29	PD‡
5	W/G/5	7	Bone marrow, multiple skeletal metastases, abdominal tumor, lymphadenopathy	100	800	42	PR‡
	(Course 2)	8	Same as above	100	800	32	MR‡
	(Course 3)	11	Hepatomegaly in addition to above	150	600	15	PD‡
6	W/G/3	7	Tumor (chest wall), multiple skeletal metastases, lymphadenopathy	100	800	37	PR‡
	(Course 2)	8	Same as above	100	200	8	NE‡
7	W/G/6	10	Bone marrow	100	600	31	PR‡
	(Course 2)	11	Hepatomegaly in addition to above	100	300	21	NE‡
8	W/B/3	11	Bone marrow, tumor (mandible), abdominal tumor, hepatomegaly, lymphadenopathy	100	200	4	NE
9	W/B/3	9	Bone marrow, tumors (orbit, skull), multiple skeletal metastases, mediastinal tumor, lymphadenopathy	100	600	22	PD‡
10	W/B/9	14	Bone marrow, tumor (skull), abdominal tumor, hepatomegaly, lymphadenopathy	100	300	12	NE‡
11	W/G/5	19	Bone marrow, tumor (mandible), multiple skeletal metastases	100	800	43	SD‡
12	W/G/2	6	Tumors (skull, orbit), mediastinal and abdominal tumors, lymphadenopathy	100	400	15	PD
13	W/G/4	10	Bone marrow, abdominal tumor, lymphadenopathy	100	200	8	NE

*W = white, G = girl, B = boy.
†NE = not evaluable, PD = progressive disease, PR = partial response, MR = mixed response, SD = stable disease.
‡Subjective improvement after therapy.

Patient Selection

Admission to the protocol was contingent upon informed consent by the parents and upon adequate kidney and liver function in the patients, as described earlier (12). All patients had advanced disease previously treated with cyclophosphamide, adriamycin, and vincristine. Twelve of the children had also received radiation therapy and 11 had metastatic bone marrow involvement when admitted to the study. The only antitumor therapy other than VM-26 given during the study was local palliative ^{60}Co radiotherapy to two patients. Changes in tumors within the radiation ports of these patients were not considered in the evaluation of drug effects.

Drug Preparation and Administration

Because hypotension has been reported to follow rapid iv administration (6), the drug was diluted to a concentration of 1 mg/ml in a solution of 5% glucose-1/3 N saline, and was then slowly infused iv over a period of 30–45 minutes.

The study design consisted of 4-week courses of VM-26 administered twice weekly in doses of 100

mg/m² (eight total doses per course). Antitumor therapy was discontinued when the disease became hopelessly progressive. Consequently, full courses of VM-26 chemotherapy were administered to only four patients.

Evaluation of Responses (Table 1)

Because solid tumors rarely respond rapidly to effective antitumor drugs, we arbitrarily required that the patients receive a minimum cumulative dose of 400 mg/m² of VM-26 over at least a 2-week period to be considered in the evaluation of antitumor effects. Categories of responses were defined as: (*a*) *Complete*—disappearance of all tumors, as determined by physical and roentgenographic examinations, and the absence of tumor cells in bone marrow with full reinstatement of normal proportions of bone marrow cell populations, as demonstrated by microscopic examination of marrow aspirates or biopsied specimens. (*b*) *Partial*—> 50% decrease in the size of the tumor and > 50% reduction in the proportion of tumor cells in the bone marrow. (*c*) *Mixed*—decrease in the size of some tumors and persistence of others. (*d*) *Stable disease*—no changes in tumors or bone marrow status. (*e*) *Progressive disease*—increase in either the size of the tumor or the extent of bone marrow involvement or both. Patients with mixed, stable, or progressive disease after VM-26 therapy were considered to be nonresponsive.

Regardless of the total dose received, all patients were evaluated for subjective responses and acute toxicity. Subjective responses included relief of pain, decrease of disease-related fever, and increase in appetite and physical activity. To monitor toxicity, all patients were examined twice weekly. The following laboratory tests were performed before therapy and at weekly intervals during the study: hemoglobin concentration, wbc, differential, and platelet counts, BUN, creatinine, uric acid, bilirubin, SGOT, LDH, alkaline phosphatase, albumin, globulin, amylase, lipase, urinalysis, and the determination of coagulation factors. Chest and skeletal roentgenograms as well as bone marrow examinations were obtained before the study and at 2–4-week intervals thereafter.

Hematologic toxicity could not be assessed in every patient because of severe impairment of marrow functions due to tumor infiltration. For this reason, evaluation was restricted to patients with an initial hemoglobin concentration > 10 g/100 ml, a wbc count > 2000/mm³, and a platelet count > 100,000/mm³. During treatment, leukopenia was defined as a wbc count < 2000/mm³ and thrombocytopenia was defined as a platelet count < 100,000/mm³.

RESULTS

Tumor Responses (Table 1)

Seven of the 13 patients met the criteria for evaluation of antitumor effectiveness of VM-26. Three had partial responses and one had stable disease, and all four completed a full course of chemotherapy (cumulative dose of 800 mg/m²). The other three patients had progressive disease and received between 400 and 700 mg/m² of VM-26. Patient 5, who showed the most striking improvement, had > 50% reduction in the size of supraclavicular nodes and in an abdominal mass, and a complete disappearance of tumor cells in the bone marrow with a return of normal hematopoiesis.

The second patient (No. 6), who did not present with bone marrow disease, had > 50% reduction of an extensive chest-wall mass that was continuous with the axillary lymph nodes on the left side. The third patient (No. 7) began VM-26 chemotherapy with complete tumor cell replacement of bone marrow; she had no other sites of disease for evaluation. After therapy, normal proportions of marrow cells reappeared and tumor cells were reduced to 15% of the total marrow cell population.

Of the six children not meeting the criteria for evaluation of antitumor effects by VM-26, five had their treatment interrupted because of progressive disease. One of these six patients (No. 10) had a reduction of tumor within the liver in addition to a subjective response, but died of uncontrolled bleeding resulting from cyclophosphamide-associated cystitis. An autopsy indicated massive tumor necrosis within the liver.

Response to Second and Third Courses of VM-26

After noting that brief interruptions of treatment resulted in rapid recurrence of tumor, we administered a second course of VM-26 to the three patients who had partial responses. The only patient (No. 5) to complete the second course had a mixed response characterized by a reduction in the size of the tumor-involved lymph nodes. She later received a third course of chemotherapy at a higher dose (150 mg/m²/dose) over a 2-week period, but had no further response. Of the other two children who began second courses of VM-26, one died with progressive disease and the other was placed on another program of chemotherapy because of progressive disease.

TABLE 2.—Hematologic toxicity following administration of VM-26

Patient No.	Chemotherapeutic course	Changes in wbc count (cells/mm³)				Changes in platelet count (cells/mm³)			
		Initial count	Leukopenia (Day)			Initial count	Thrombocytopenia (Day)		
			Onset	Nadir	Recovery		Onset	Nadir	Recovery
1	First	3700	1800 (7)	1800 (7)	2100 (10)	24,000	72,000 (10)	72,000 (10)	None*
2	First	1800		1400 (14)	None†	18,000		18,000 (14)	None†
3	First	4900	1700 (7)	1300 (17)	2800 (28)	25,000	6000 (14)	6000 (14)	None*
4	First	2400	1900 (3)	500 (10)	None	460,000	38,000 (24)	22,000 (24)	None†
5	First	3300	1500 (20)	1500 (20)	3200 (32)	336,000	None		
	Second	4400	None	—	—	200,000	None		
	Third	4200	1800 (8)	1000 (17)	None*	23,000	20,000 (11)	20,000 (11)	None*
6	First	11,000	None	—	—	600,000	None		
	Second	13,600	1600 (8)	1600 (8)	2600 (15)	575,000	None		
7	First	4300	None	—	—	80,000	52,000 (4)	10,000 (7)	56,000 (21)
	Second	3100	None	—	—	20,000	16,000 (8)	16,000 (8)	None†
8	First	12,900	None	—	—	78,000			84,000 (6)†
9	First	4500	1300 (12)	600 (16)	None†	50,000	44,000 (5)	2000 (22)	None†
10	First	5300	1500 (10)	700 (12)	None†	300,000	14,000 (10)	6000 (12)	None†
11	First	4100	1800 (12)	500 (22)	None*	220,000	44,000 (15)	8000 (36)	None*
12	First	2400	800 (15)	800 (15)	4000 (20)	18,000		14,000 (11)	136,000 (20)
13	First	5600	1200 (5)	1200 (5)	None†	60,000	6000 (4)	2000 (5	None†

*Began new chemotherapy schedule.
†Death within 7 days of nadir.

Subjective Improvement

Subjective responses were observed in nine of the 13 children. Most significant was the rapid disappearance of pain within 24–48 hours after initiation of therapy. The group with symptomatic improvement included all four patients who received 800 mg/m², three of the four patients who received between 400 and 700 mg/m², but only two of the six patients who received < 400 mg/m². The children who received more than one course of chemotherapy experienced subjective responses throughout their treatment.

Toxicity (Table 2)

A total of 85 doses of VM-26 were administered during this study. None of the 13 children developed hypotension, compromised liver or kidney function, or other adverse side effects. Two patients had episodes of vomiting after the administration of VM-26. Swelling and tenderness of the forearm developed around the site of VM-26 infusion in one patient but subsided within 24 hours. Another patient developed bacterial pneumonia that responded to specific antimicrobial therapy.

Hematologic toxicity was difficult to evaluate as evident from the clinical status of the patient population. Upon admission to the protocol, all patients were anemic and therefore could not be evaluated for alteration of rbc production during the administration of VM-26. Chemotherapy, however, did not increase the requirement for blood product transfusions. Nine of 12 children developed leukopenia within the first 3–20 days of treatment. The median latency of the nadir was 16 days with recovery in only four of the nine children. For the five patients whose wbc counts did not recover, four died and one began a different course of chemotherapy. Four of the six patients with normal platelet counts developed thrombocytopenia between 10 and 24 days of treatment. The median latency of the nadir was 18 days. Of these four patients, one child died as a result of progressive disease and two received a different chemotherapeutic program before adequate recovery of their platelet count. Another child (patient 10) developed severe bleeding due to cyclophosphamide-induced cystitis before the administration of VM-26, but the volume of blood loss did not increase with the onset of thrombocytopenia. Of the seven children entering this study with thrombocytopenia, two had in-

creases in their platelet counts while receiving VM-26. Except for patient 10, petechiae, ecchymoses, and mucosal bleeding were not observed in the study group.

DISCUSSION

VM-26 produced a > 50% reduction of tumor in three children with disseminated neuroblastoma refractory to conventional chemotherapy. The full therapeutic potential of the compound probably was not realized because only seven of 13 patients received sufficient chemotherapy to qualify for antitumor evaluation. Treatment in five of six children was not continued due to progressive disease, while toxicity was never a limiting factor for the administration of therapy. We treated this study group, with the exception of the first patient, with doses of 100 mg/m²/dose because in earlier trials 50 mg/m²/dose did not produce significant toxic effects or clinical response (12).

Because oncolytic responses in patients with solid tumors are rarely elicited as early as in patients with leukemia or lymphomas, we arbitrarily required that a minimum total dose of 400 mg/m² be given within 2 weeks before assessing the antitumor activity of VM-26. These criteria for dose and time of treatment proved valid since the responses did, in fact, occur in patients who received 800 mg/m² and appeared during the third or fourth week of VM-26 therapy, never earlier.

Peculiar to disseminated neuroblastoma is the frequent occurrence of extensive bone marrow involvement; therefore, the most encouraging response was the disappearance of bone marrow tumor with regeneration of normal cells. This was especially notable in patient 5, a child with bone marrow involvement since diagnosis which had not disappeared with previous administration of cyclophosphamide, adriamycin, or vincristine. The presence of extensive bone marrow disease in most of our patients precluded direct assessment of VM-26 toxicity on hematopoiesis. In general, patients with severe marrow involvement developed leukopenia earlier and to a greater degree than did patients with less extensive involvement, but two of them were able to tolerate cumulative dosages of > 1000 mg/m² of VM-26 without limiting toxicity.

The results in this study using VM-26 alone are comparable to those previously obtained with vincristine (13,14) or DTIC (15). Today, both of these drugs are given in the initial treatment of neuroblastoma although as single agents they rarely produced complete remissions. Finklestein et al (15) reported three partial remissions among 18 children treated with DTIC. James and George (13) also reported three partial remissions in seven patients treated with the vinca alkaloid, two occurring in previously untreated patients. Windmiller et al (14) treated 20 children with vincristine alone; in the 13 evaluable patients, two partial responses and one complete response in a 4-month-old infant were observed.

Having demonstrated effects of VM-26 in patients with late stages of disease, it seems warranted to use this compound earlier. As with other agents, the administration of VM-26 as a single drug would probably not suffice. This is suggested by our observations in the three children who had drug-sensitive disease but who developed rapid tumor enlargement after brief interruptions of treatments. Moreover, the failure of second courses (table 1) of VM-26 to improve the initial results may indicate that, for a sustained tumor response, VM-26 should be used in combination with other agents known to be effective against neuroblastoma.

Pouillart et al (16,17) and Eckhardt et al (18) have demonstrated the effectiveness of VM-26 in combination chemotherapy in other human malignancies. Our studies against L1210 murine leukemia emphasized the superiority of VM-26, or its analog VP-16-213, when combined with cytosine arabinoside (19).

At our institution, cell kinetic studies performed on marrow tumor cells of children with neuroblastoma have demonstrated that after cyclophosphamide therapy the number of cells traversing the cell cycle from S phase through mitosis is increased (20). Since the cytotoxic effect of VM-26 is greatest on cells in the G_2 phase (21,22), a sequential combination of both agents might be advantageous in patients whose tumor cells are sensitive to cyclophosphamide. In addition, a combination with adriamycin may be effective (23). Preliminary studies presently are being performed to assess the value and to determine the preferred scheduling of the administration of VM-26 and adriamycin in children with disseminated neuroblastoma.

REFERENCES

1. GREEN AA, HUSTU HO, and KUMAR M. The response of neuroblastoma to sequential low-dose cyclophosphamide (cyclo) and adriamycin (adria) therapy. Proc Am Assoc Cancer Res and ASCO 17:120, 1976.
2. THURMAN WG, FERNBACH DG, and SULLIVAN MP. Cyclophosphamide therapy in childhood neuroblastoma. N Engl J Med 270:1336–1340, 1964.
3. TAN C, ROSEN G, GHAVINI F, ET AL. Adriamycin (NSC-123127) in pediatric malignancies. Cancer Chemother Rep Part 3, vol 6(2):259–266, 1975.
4. STÄHELIN H. 4'-Demethylepipodophyllotoxin thenylidene

glucoside (VM-26) a podophyllum compound with a new mechanism of action. Eur J Cancer 6:303–311, 1970.

5. AVERY TL, ROBERTS D, and PRICE RA. Delayed toxicity of 4′-demethylepipodophyllotoxin 9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside) (NSC-122819; VM-26) in mice. Cancer Chemother Rep 57:165–173, 1973.
6. MUGGIA FM, SELAWRY OS, and HANSEN HH. Clinical studies with a new podophyllotoxin derivative, epipodophyllotoxin, 4′-demethyl-9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside) (NSC-122819). Cancer Chemother Rep 55:575–581, 1971.
7. DOMBERNOWSKY P, NISSEN NI, and LARSEN V. Clinical investigation of a new podophyllum derivative, epipodophyllotoxin, 4′-demethyl-9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside) (NSC-122819), in patients with malignant lymphomas and solid tumors. Cancer Chemother Rep 56:71–82, 1972.
8. EUROPEAN ORGANIZATION FOR RESEARCH OF THE TREATMENT OF CANCER. Clinical screening of epipodophyllotoxin VM-26 in malignant lymphomas and solid tumours. Br Med J 2:744–748, 1972.
9. SONNTAG RW, SENN HJ, NAGEL G, ET AL. Experience with 4′-demethylepipodophyllotoxin, 9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside); VM-26; NSC-122819 in the treatment of malignant lymphosis. Eur J Cancer 10:93–98, 1974.
10. MATHÉ G, SCHWARZENBERG L, POUILLART P, ET AL. Two epipodophyllotoxin derivatives, VM-26 and VP-16213, in the treatment of leukemias, hematosarcomas, and lymphomas. Cancer 34:985–922, 1974.
11. SKLANSKY BD, MANN-KAPLAN RS, REYNOLDS AF, JR, ET AL. 4′-Demethylepipodophyllotoxin-β-D-thenylidene-glucoside (PTG) in the treatment of malignant intracranial neoplasms. Cancer 33:460–467, 1974.
12. RIVERA G, AVERY T, and PRATT C. 4′-Demethylepipodophyllotoxin 9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside) (NSC-122819; VM-26) and 4′-demethylepipodophyllotoxin 9-(4,6-*O*-ethylidene-β-D-glucopyranoside) (NSC-141540; VP-16-213) in childhood cancer: preliminary observations. Cancer Chemother Rep 59:743–749, 1975.
13. JAMES D, and GEORGE P. Vincristine in children with malignant solid tumors. J Pediatr 64:534–541, 1964.
14. WINDMILLER J, BERRY DH, VIETTI T, ET AL. Vincristine sulfate in the treatment of neuroblastoma in children. Am J Dis Child 111:75–78, 1966.
15. FINKLESTEIN JZ, ALBO V, ERTEL I, ET AL. 5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide (NSC-45388) in the treatment of solid tumors in children. Cancer Chemother Rep 59:351–357, 1975.
16. POUILLART P, SCHWARZENBERG L, AMIEL JL, ET AL. Combinaisons chimiotherapiques de drogues se potentialisant. III. Application aux tumeurs primitives du systeme nerveux central. Nouv Presse Med 4:721–724, 1975.
17. POUILLART P, WEINER R, SCHWARZENBERG L, ET AL. Combination chemotherapy based on a model of cell recruitment by partial synchronization. Med Pediatr Oncol 1:123–134, 1975.
18. ECKHARDT S, DOBRENTEY E, and BODROGI I. Results obtained with combination therapy of VM-26, Natulan and prednisolone in generalized Hodgkin's disease. Chemotherapy 21:248–254, 1975.
19. RIVERA G, AVERY T, and ROBERTS D. Response of L1210 to combinations of cytosine arabinoside and VM-26 or VP16-213. Eur J Cancer 11:639–647, 1975.
20. HAYES FA, GREEN AA, and MAUER AM. The correlation of cell kinetics and clinical response to chemotherapy in disseminated neuroblastoma. Cancer Res, Oct 1977. In press.
21. KRISHAN A, PAIKA K, and FREI E, III. Cytofluorometric studies on the action of podophyllotoxin and epipodophyllotoxins (VM-26, VP16-213) on cell cycle traverse of human lymphoblasts. J Cell Biol 66:521–530, 1975.
22. MISRA NC, and ROBERTS D. Inhibition by 4′-demethyl-epipodophyllotoxin 9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside) of human lymphoblast cultures in G_2 phase of the cell cycle. Cancer Res 35:99–105, 1975.
23. GREEN AA, HAYES FA, and RIVERA G. Epipodophyllotoxin VM-26 in the treatment of neuroblastoma. Proc Am Assoc Cancer Res and ASCO 18:283, 1977.

Implications of the ^{99m}Tc Diphosphonate Bone Scan on Treatment of Primary Breast Cancer [1,2]

D. L. Citrin,* D. C. Tormey, and P. P. Carbone [3]

SUMMARY

The ^{99m}Tc diphosphonate scan is important in the study of primary breast cancer. The short-term prognosis for scan-positive patients is poor. Serial bone scans provide a safe and reliable prognostic index in patients following mastectomy. By correlating the scan with biochemical tumor markers, a matrix of diagnostic tests may be developed that is capable of identifying high-risk patients who may benefit from systemic therapy.

[Cancer Treat Rep 61:1249–1252, 1977]

Most patients with primary carcinoma of the breast with metastatic disease in the axillary nodes (N+) are not cured by their local treatment. Although surgery with or without radiation therapy is frequently successful in adequately controlling local and regional disease, only one N+ patient in four is alive 10 years after primary treatment (1). The recognition that most N+ patients have clinically occult micrometastases which have disseminated widely prior to clinical presentation has led to the use of systemic chemotherapy as an adjuvant to mastectomy during the postoperative period in patients whose nodal status indicates a high risk of developing metastatic disease. The preliminary results of prospective randomized clinical trials of adjuvant chemotherapy show a significant reduction in recurrence rates in premenopausal patients compared with untreated controls although investigators urge caution in assessing the results until more prolonged followup is available (2,3).

An alternative approach to the use of adjuvant chemotherapy in all moderately high-risk (N+) patients is to attempt to identify and treat those patients with a particularly high risk of developing metastatic disease. In this context, biochemical and immunologic tests reflecting abnormal or inappropriate tumor-cell metabolism are of value in certain selected tumors (4,5), and tumor markers are now being evaluated in human breast cancer with encouraging results (6).

One area where progress has recently been made is in the identification of clinically occult metastases in bone. The skeleton is the most common site for metastatic disease in breast cancer, with an autopsy incidence of $> 80\%$ (7) and a first recurrence incidence of $> 50\%$ (8). The conventional radiograph is an insensitive method of detecting bone metastases (9) and it is now well established that radioisotope bone scans are more accurate (10,11). The technique is particularly appropriate to the study of breast cancer (12–15), especially since most bone metastases in this disease are readily visible scintigraphically.

Isotope bone scans have been used for many years in patients presenting with surgically "curable" breast cancer to demonstrate the presence of clinically occult skeletal metastases. These studies, which employed early scanning equipment and the now obsolete bone scanning agents ^{85}strontium (16), ^{87m}strontium (17), and ^{18}fluorine (18), demonstrated that a significant minority (16%–24%) of patients with breast cancer apparently localized to the breast and axillary nodes had clinically unsuspected skeletal metastases demonstrated by isotope bone scanning. A recent long-term followup of two of these groups of patients has shown that the subsequent incidence of clinically and radiologically evident bone metastases was higher in the scan-positive than in the scan-negative patients (19,20).

[1]Received Mar 21, 1977; revised May 16, 1977; accepted May 23, 1977.

[2]Supported by Medical Research Council Travelling Fellowship in Cancer, 1976 (Dr. Citrin).

[3]Department of Human Oncology, University of Wisconsin Hospital, Madison.

*_Reprint requests to:_ D. L. Citrin, MB, ChB, PhD, MRCP, Department of Human Oncology, University of Wisconsin Hospital, 1300 University Ave, Madison, Wis 53706.

It is important that those who are not primarily involved in nuclear medicine practice, but rely on the bone scan to provide important staging information, are aware of how profoundly the technique has changed in the past few years and the significance of this as it relates to clinical practice. The recent introduction of the ^{99m}Tc-labeled phosphate and diphosphonate compounds has made high-quality bone scans available in all hospitals, including those with limited nuclear medicine facilities. The bone scan produced by ^{99m}Tc diphosphonate is significantly different from that obtained with strontium and fluorine. There is, therefore, a need to reassess the clinical significance of the bone scan in terms of staging primary breast cancer (20).

The preliminary results of three studies with ^{99m}Tc-labeled agents have confirmed the results previously obtained with strontium and fluorine (21–23). In these recent studies, the frequency of positive bone scans in patients with clinically apparently localized breast cancer (stages I and II) with negative radiologic surveys ranged from 6% to 30% (table 1). Of particular importance is the demonstration that a positive ^{99m}Tc bone scan, whether initially positive or due to conversion of a previously negative study, is associated with a poor short-term prognosis (23).

The bone scan is a more sensitive indicator of skeletal disease than the radiograph, and with appropriate radiographic correlation (particularly of solitary lesions [24]), the incidence of false-positive scans due to the presence of benign disease is acceptably low. The use of skeletal radiographs in preference to bone scans as the routine screening investigation for bony metastases is, therefore, no longer tenable. Accurate pretreatment staging is of prime importance in any trial of cancer treatment. An isotope bone scan should be considered an essential part of the initial work-up of patients entered into trials of post-mastectomy adjuvant therapy.

Of probably greater importance than the ability to identify a minority of patients with bone metastases at the time of mastectomy is the unique opportunity now afforded to identify progression of skeletal metastases in entirely asymptomatic patients during the "disease-free" interval after mastectomy. The radiation dose to the patient of a ^{99m}Tc diphosphonate bone scan is of a similar magnitude to that of a radiologic skeletal survey (25). The acceptably low radiation dose from ^{99m}Tc diphosphonate, together with its ready availability, now makes it possible for the first time to perform *repeated* bone scans in the same patient. Serial bone scans have demonstrated that conversion of a negative bone scan to positive may occur in the absence of clinical or radiologic evidence of local recurrence or distant metastases. Rapid scan conversion indicates progression of occult skeletal metastases and is associated with a particularly poor short-term prognosis (23).

By employing a pretreatment bone scan and repeated scans at 6-month intervals, it is likely that during the first 2–3 years after mastectomy, approximately 30% of patients presenting with a $T_{1-2}N_1$ tumor will be scan-positive, either initially or by conversion (table 2) (23). This estimate tends to be confirmed by the results of Bonadonna et al, where the most common site of recurrence was the skeleton. It is noteworthy that in this study, of 27 patients with radiologically evident metastases, no fewer than ten were entirely asymptomatic and "could be detected only by systemic (radiologic) bone survey" (3). It is very probable that in patients receiving adjuvant therapy, additional cases of treatment failure could be detected at an earlier date by the use of followup bone scans instead of radiographs.

It should be stressed here that we do not advocate the total exclusion of skeletal radiology from the investigation of patients with breast cancer, but

TABLE 1.—^{99m}Tc phosphate and diphosphonate bone scan in primary breast cancer

Total No. of patients	Mean followup time	Scan positive at time of primary treatment (%)	No. of patients with repeat scan	No. with scan converter (%)	Total patients with positive scan (%)	No. confirmed as metastatic (%)	Ref No.
164	12 mos	10 (6)	52	10/52 (19)	20 (12)	14/20	21
78	Not stated	23 (30)	0	—	23 (30)	10/23	22
75	30 mos	10 (13)	74	15/65 (23)	25 (33)	12/25	23*
317		43 (14)	126	25/117 (21)	68 (21)	26/68 (53)	

*Updated data.

TABLE 2.—% of patients with a positive bone scan after mastectomy

Time scan taken	No. of patients scanned	% positive
At mastectomy	75	13
12 mos	74	24
24 mos	64	28
36 mos	39	33

merely the replacement of the radiograph by the bone scan as the study of choice for pretreatment screening for occult metastases. During followup, serial bone scans should be performed at regular intervals rather than repeated radiologic surveys. The bone scan should no longer be considered as a "super-radiograph" used to confirm the presence of clinically suspected bone metastases when radiographs are normal. The bone scan is a tumor marker, capable of detecting the presence of, and documenting by repeated study, the biologic behavior of truly occult micrometastases, with a potential sensitivity probably approaching that of currently available biochemical and immunologic markers.

If, as seems likely, the bone scan is accepted in this role, the opportunity will become available for detailed studies of the precise diagnostic and prognostic significance of serial bone scans (positive *and* negative) in patients receiving adjuvant chemotherapy, thereby increasing the information obtainable from present adjuvant studies (figs 1 and 2). The relationship of the bone scan to clinical and pathologic factors, and to established (and potential) tumor markers, can be evaluated and related to clinical course, therapeutic response, and ultimately, survival. In this way, the bone scan can be developed as one component of a matrix of diagnostic tests designed to provide prognostic information leading to earlier and more rational therapeutic programs in individual patients.

1. No method available for monitoring therapeutic effect, except endpoint analysis
2. Arbitrary discontinuation of treatment
3. No biologic information concerning reasons for treatment failures

FIGURE 1.—Present status of adjuvant chemotherapy studies.

1. Identify particularly high-risk patients
2. Demonstrate objective response to therapy
3. Indicate need for change of adjuvant therapy
4. Provide a rationale for stopping therapy
5. Provide a method for posttherapy followup
6. Correlate scan results with other tumor markers

FIGURE 2.—Potential advantages of serial bone scans in studies of adjuvant chemotherapy in breast cancer.

REFERENCES

1. FISHER B, SLACK N, KATYCH D, ET AL. Ten year follow-up of breast cancer patients in a cooperative clinical trial evaluating surgical adjuvant chemotherapy. Surg Gynecol Obstet 140:528–534, 1975.
2. FISHER B, CARBONE PP, ECONOMOU SG, ET AL. L-Phenylalanine mustard (L-PAM) in the management of primary breast cancer: a report of early findings. N Engl J Med 292:117–122, 1975.
3. BONADONNA G, BRUSAMOLINO E, VALAGUSSA PL, ET AL. Combination chemotherapy as an adjuvant treatment in operable breast cancer. N Engl J Med 294:405–410, 1976.
4. RUDNICK P, and ODELL WD. In search of a cancer. N Engl J Med 284:405–408, 1971.
5. HOLYOKE D, REYNOSO G, and CHU TM. Carcinoembryonic antigen (CEA) in patients with carcinoma of the digestive tract. Ann Surg 176:559–564, 1972.
6. TORMEY DC, WAALKES TP, AHMANN D, ET AL. Biological markers in breast carcinoma. Incidence of abnormalities of CEA, hCG, three polyamines, and three minor nucleosides. Cancer 35:1095–1100, 1975.
7. JAFFE HL. Tumors and Tumorous Conditions of the Bones and Joints. London, Henry Kimpton, 1958, p 600.
8. BRUCE J, CARTER DC, and FRASER J. Patterns of recurrent disease in breast cancer. Lancet 1:433–435, 1970.
9. BORAK J. Relationship between the clinical and roentgenological findings in bone metastases. Surg Gynecol Obstet 75:599–604, 1942.
10. PISTENMA DA, MCDOUGALL IR, and KRISS JP. Screening for bone metastases. Are only scans necessary? JAMA 231:46–50, 1975.
11. TOFE AJ, FRANCIS MD, and HARVEY WJ. Correlation of neoplasms with incidence and local ratio of skeletal metastases: an analysis of 1355 diphosphonate bone scans. J Nucl Med 16:986–989, 1975.
12. GALASKO CSB, WESTERMAN B, LI J, ET AL. Use of the gamma camera for early detection of osseous metastases from mammary cancer. Br J Surg 55:613–615, 1968.
13. RUBIN P, and CICCIO S. Status of bone scanning for bone metastases in breast cancer. Cancer 24:1338–1344, 1969.
14. HOPKINS GB, and KRISTENSEN KAB. Whole body skeletal scintiphosography in the detection of occult metastatic breast carcinoma. Calif Med 119:10–13, 1973.
15. CITRIN DL, BESSENT RG, GREIG WR, ET AL. The application of the ^{99m}Tc phosphate bone scan to the study of breast cancer. Br J Surg 62:201–204, 1975.
16. SKLAROFF DM, and CHARKES ND. Bone metastases from breast cancer at the time of radical mastectomy. Surg Gynecol Obstet 127:763–768, 1968.
17. HOFFMAN HC, and MARTY R. Bone scanning. Its value in the preoperative evaluation of patients with suspicious breast masses. Am J Surg 124:194–199, 1972.
18. GALASKO CSB. The detection of skeletal metastases from mammary cancer by gamma camera scintigraphy. Br J Surg 56:757–764, 1969.
19. GALASKO CSB. The significance of occult skeletal metastases, detected by skeletal scintigraphy, in patients with otherwise apparently "early" mammary carcinoma. Br J Surg 63:694–696, 1975.

20. SKLAROFF RB, and SKLAROFF DM. Bone metastases from breast cancer at the time of.radical mastectomy as detected by bone scan. Eight year follow-up. Cancer 38:107–111, 1976.
21. LENTLE BC, BURNS PE, and DIERICH H. Bone scintiscanning in the initial assessment of carcinoma of the breast. Surg Gynecol Obstet 141:43–47, 1975.
22. ROBERTS JG, GRAVELLE IH, BAUM M, ET AL. Evaluation of radiography and isotopic scintigraphy for detecting skeletal metastases in breast cancer. Lancet 1:237–239, 1976.
23. CITRIN DL, FURNIVAL CM, BESSENT RG, ET AL. Radioactive technetium phosphate bone scanning in preoperative assessment and follow-up study of patients with primary cancer of the breast. Surg Gynecol Obstet 143:360–364, 1976.
24. CORCORAN RJ, THRALL JH, KYLE RW, ET AL. Solitary abnormalities in bone scans of patients with extra osseous malignancies. Radiology 121:663–667, 1976.
25. CITRIN DL. The use of radioactive nuclides in the investigation of malignant disease. PhD Thesis, University of Glasgow, vol 2, pp 15–16, 1975.

Effects of Adriamycin and Actinomycin D on Nucleolar Morphology: A Simple Biologic Assay[1,2,3]

Karel Smetana, Jerome Merski, Yerach Daskal, Rose K. Busch, and Harris Busch[4,*]

Both electron and light microscopic studies have shown that distinct changes occur in the distribution of nucleolar RNP components after treatment of cells with various inhibitors of RNA synthesis (1–6). These alterations have been characterized by the segregation of nucleolar components, increased size and number of fibrillar centers, loosening or unraveling of nucleolonemas, and formation of ring-shaped nucleoli.

In the present study, toluidine blue staining of smear preparations was used to investigate the changes in the distribution of nucleolar RNP produced by actinomycin D and adriamycin in Novikoff hepatoma cells. This simple staining procedure permits selective demonstration of RNA-containing structures in smeared cells and does not stain chromatin. These preparations were analyzed for the types of nucleolar aberrations that occurred in Novikoff hepatoma cells following antibiotic therapy and the dose-effect relationships for two agents, adriamycin and actinomycin D. Quantitation of the nucleolar aberrations detected by this technique provides a simple, rapid screening method for evaluating the antinucleolar activity of some antitumor antibiotics.

MATERIALS AND METHODS

Ten groups of male Holtzman rats (three animals per group) bearing 6-day-old Novikoff hepatoma ascites tumors were treated with either adriamycin (Adria Laboratories, Wilmington, Del) or actinomycin D (Calbiochem, Los Angeles, Calif). Both agents were administered ip. The doses of adriamycin used ranged from 0.15 to 10.0 mg/kg; actinomycin D was given in doses of 0.05–1.0 mg/kg. Control animals received equivalent volumes of 0.9% NaCl.

One hour after treatment the animals were killed by decapitation and the ascites fluid was removed. Five to seven smear preparations were made for each animal from aliquots of the tumor fluid. The unfixed, smeared Novikoff hepatoma cells were then stained for approximately 5 minutes with a 0.05% solution of toluidine blue (LaChema, Czechoslovakia) buffered with McIlvain buffer at pH 5. This procedure selectively stains nucleoli and other RNP-containing structures and does not stain chromatin (7). A minimum of three slides were scored for each animal with an average of 125 cells evaluated on each slide. The number and percentage of nucleoli were determined in various categories including (*a*) compact nucleoli, (*b*) nucleoli with distinct or separated nucleolonemas and/or large light areas, (*c*) ring-shaped nucleoli, and (*d*) micronucleoli.

The percentage of viable cells was determined for each sample by supravital staining with a 0.4% solution of trypan blue. In all instances not $< 90\%$ of the treated cells were found to be viable by this criterion.

RESULTS

Structure of Nucleoli in Untreated Cells

Novikoff hepatoma cells contain several different types of nucleoli which differed with respect to the distribution of their RNP components. Most nucleoli were compact and of relatively uniform density (figs 1a and 2a). These nucleoli varied in diameter from 1.3 to 14 μm and in shape from irregular to round (figs 1a–1d). Nucleoli with large light areas or distinct nucleolonemas were less frequently observed. Ring-shaped nucleoli and micronucleoli (figs 1c and 1d) were rare (table 1).

Structure of Nucleoli in Treated Cells

Administered individually, both adriamycin and actinomycin D decreased the percentage of compact nucleoli observed in the control cells (tables 1 and 2). This decrease was accompanied by an increase

[1]Received Feb 16, 1977; revised June 14, 1977; accepted June 20, 1977.

[2]Supported by Public Health Service grant CA-10893 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare, the Davidson Fund, the Wolff Memorial Foundation, and gifts from Dr. and Mrs. O. A. Breiling and Mrs. Jack Hutchins.

[3]Adriamycin: NSC-123127; CAS reg. No. 23214-92-8.
Actinomycin D: NSC-3053.

[4]Department of Pharmacology, Baylor College of Medicine, Houston, Tex.

**Reprint requests to:* Harris Busch, MD, Department of Pharmacology, Baylor College of Medicine, Houston, Tex 77030.

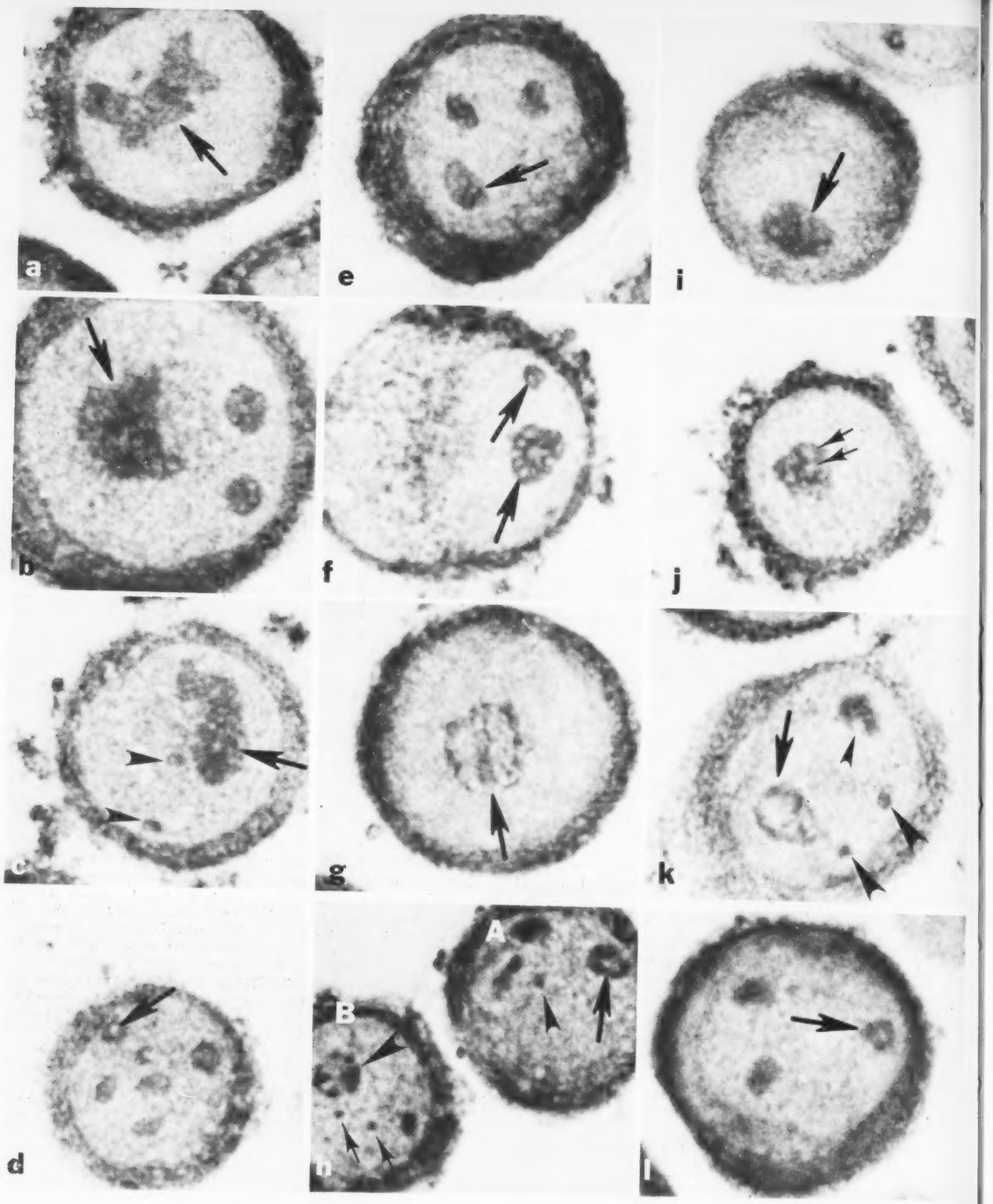

a
e
i
b
f
j
c
g
k
d
A
B
h
l

TABLE 1.—Percentages of nucleolar types found after treatment with adriamycin*

Dose (mg/kg)	Compact nucleoli	Nucleoli with large light areas	Ring-shaped nucleoli	Micronucleoli
Control	88.0 ± 2.8	3.5 ± 0.7	2.5 ± 0.7	6.0 ± 2.8
0.15	80.7 ± 2.5	6.3 ± 1.2	3.0 ± 1.0	10.0 ± 2.0
0.75	73.0 ± 6.8	11.0 ± 5.6	4.3 ± 2.1	11.3 ± 3.2
1.50	59.0 ± 5.3	16.0 ± 6.1	9.7 ± 2.1	18.7 ± 4.6
5.00	48.3 ± 2.1	21.0 ± 1.0	10.3 ± 5.1	19.3 ± 4.2
10.00	40.0 ± 14.0	26.0 ± 12.1	17.0 ± 5.0	16.7 ± 5.6

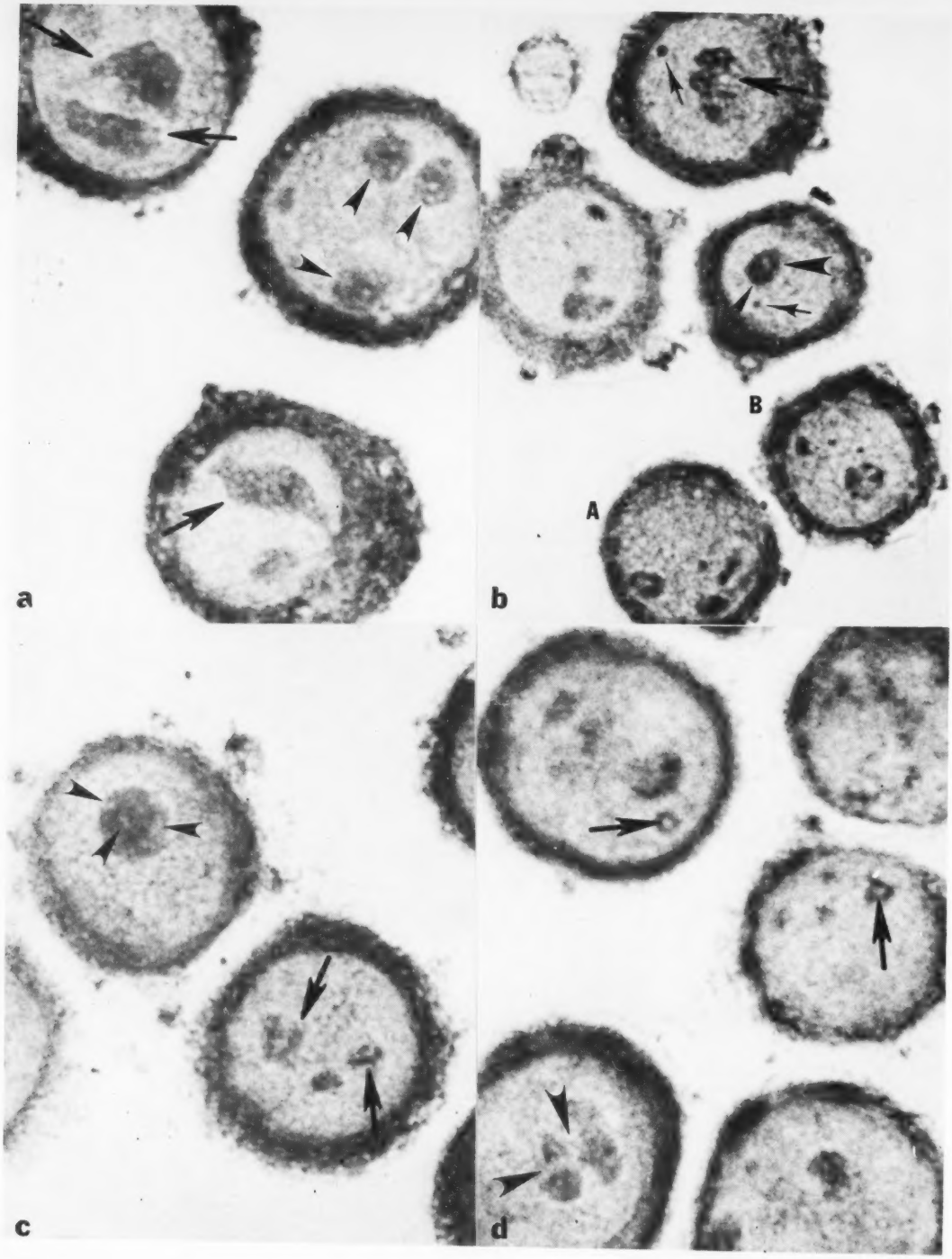
*All figures represent the average results from 3 animals.

TABLE 2.—Percentages of nucleolar types found after treatment with actinomycin D*

Dose (mg/kg)	Compact nucleoli	Nucleoli with large light areas	Ring-shaped nucleoli	Micronucleoli
Control	88.0 ± 2.8	3.5 ± 0.7	2.5 ± 0.7	6.0 ± 2.8
0.05	61.0 ± 5.1	15.0 ± 6.6	8.0 ± 2.1	16.0 ± 3.6
0.10	55.7 ± 3.2	10.7 ± 1.2	11.0 ± 3.0	21.7 ± 2.3
0.15	52.0 ± 1.4	22.0 ± 11.3	11.0 ± 5.7	15.0 ± 7.1
0.20	45.0 ± 4.5	29.0 ± 4.6	11.7 ± 3.1	14.0 ± 5.6
1.00	42.3 ± 5.8	16.7 ± 4.7	12.0 ± 3.6	27.3 ± 7.5

*All figures represent the average results from 3 animals.

FIGURE 1.—a. Untreated Novikoff hepatoma cell with a large, dense, irregularly shaped nucleolus (arrow) (× 2320). b. Untreated Novikoff hepatoma cell with 3 nucleoli. One is a large, dense, irregularly shaped nucleolus without distinct nucleolonemas (arrow). The 2 smaller nucleoli are rounded (× 2400). c. Untreated Novikoff hepatoma cell that contains 1 large, irregularly shaped, dense nucleolus (arrow) and 2 micronucleoli (pointers) (× 1600). d. Very rare example of a Novikoff hepatoma cell which contains several small dense nucleoli and 1 small ring-shaped nucleolus (arrow) (× 2320). e. 3 nucleoli with small light areas (arrow) in a Novikoff hepatoma cell after treatment with actinomycin D (1 mg/kg). These nucleoli are smaller than those usually observed in control cells. Such cells are commonly present after actinomycin D treatment (× 2500). f. Novikoff hepatoma cell containing 2 nucleoli with distinct light areas (arrow) and nucleolonemas after treatment with actinomycin D (1 mg/kg) (× 2840). g. Nucleolus of a Novikoff hepatoma cell that consists of only a few twisted and irregular separated nucleolar RNP strands (arrow) after treatment with actinomycin D (1 mg/kg) (× 2240). h. Novikoff hepatoma cell in the upper right field (A) that contains a ring-shaped nucleolus (arrow) and a micronucleolus (small pointer); the cell in the lower left field (B) contains a nucleolus with segregated RNP areas (pointer) and 2 micronucleoli (small arrows). These cells were treated with actinomycin D (1 mg/kg) (× 2500). i. Intermediate-sized nucleolus after treatment with adriamycin (10 mg/kg); the large arrow points to an area of low density (× 2240). j. Intermediate-sized nucleolus with distinct light areas (small arrows) after treatment with adriamycin (10 mg/kg) (× 2280). k. In this cell, one of the nucleoli consists only of a narrow peripheral rim and a few nucleolonemas (arrow). A second nucleolus has a prominent light area (small pointer). 2 micronucleoli are also present (pointers). This cell was treated with adriamycin (10 mg/kg) (× 2240). l. After treatment with adriamycin (10 mg/kg) 1 nucleolus of this Novikoff hepatoma cell is ring-shaped (arrow) while the other 2 are more dense and without distinguishable substructure (× 2320).


a
b
A
B
c
d

in the percentage of aberrant nucleolar forms (tables 1 and 2), especially ring-shaped nucleoli (figs 1h, 1l, 2b, and 2d), micronucleoli (figs 1h and 2b), and nucleoli with large light areas (figs 1f, 1i, 1j, and 2c). Occasionally, nucleoli consisting of only a few twisted dense RNP fibers were observed (fig 1g). In addition, segregation of nucleoli into well-separated areas (figs 1h, 1k, and 2d) and the formation of nucleolar caps (fig 2b) were occasionally seen.

DISCUSSION

This study shows that in vivo treatment of Novikoff hepatoma cells with increasing doses of either adriamycin or actinomycin D causes a progressive increase in the percentage of altered nucleolar forms observed (tables 1 and 2). These unusual nucleolar forms include nucleoli with enlarged fibrillar centers, ring-shaped nucleoli, and micronucleoli. The association of these characteristic nucleolar forms with decreased RNP content of nucleolar components as well as diminished nucleolar RNA synthesis is well documented (3–6,8). It is also well established that they occur as a result of treatment with RNA inhibitors (1,4,6). Since the antitumor activity of drugs such as adriamycin and actinomycin D is associated with their ability to inhibit RNA synthesis (9–11), quantitation of easily recognizable morphologic markers such as those used in the present study provides a rapid and simple technique for screening drugs for inhibition of RNA synthesis. In addition, this method provides a practical means of comparing the activity of analogs to their parent compounds.

REFERENCES

1. SMETANA K, and POTMĚŠIL M. Ring-shaped nucleoli in liver cells of rats after treatment with actinomycin D. Z Zellforsch 92:62–69, 1968.
2. MERSKI J, DASKAL I, and BUSCH H. Effects of adriamycin on ultrastructure of nucleoli in the heart and liver cells of the rat. Cancer Res 36:1580–1584, 1976.
3. GOLDBLATT PJ, SULLIVAN RJ, and FARBER E. Morphologic and metabolic alterations in hepatic cell nucleoli induced by varying doses of actinomycin D. Cancer Res 29:129–135, 1969.
4. POTMĚŠIL M, and SMETANA K. The inhibitory effect of chromomycin A_3 on the RNA synthesis and the formation of ring-shaped nucleoli in cell cultures. Folia Biol (Praha) 14:132–139, 1968.
5. LOVE R, and SORIANO RZ. Correlation of nucleolini with fine structural nucleolar constituents of cultural normal and neoplastic cells. Cancer Res 31:1030–1037, 1971.
6. POTMĚŠIL M, and SMETANA K. Significance of ring-shaped nucleoli and micronucleoli in human lymphocytes. Folia Biol (Praha) 15:300–305, 1969.
7. SMETANA K, LEJNAR J, and POTMĚŠIL M. A further contribution to the demonstration of RNA and nucleoli of blood cells in smear preparations. Folia Haematol (Leipz) 91:381–394, 1969.
8. SIMARD R. Specific nuclear and nucleolar ultrastructural lesions induced by proflavin and similarly acting antimetabolites in tissue culture. Cancer Res 26:2316–2328, 1966.
9. GOLDBERG IH, RABINOWITZ M, and REICH E. Basis of actinomycin action. I. DNA binding and inhibition of RNA-polymerase synthesis reactions by actinomycin. Proc Natl Acad Sci USA 48:2094–2101, 1962.
10. DIMARCO A. Adriamycin (NSC-123127): mode and mechanism of action. Cancer Chemother Rep Part 3, vol 6(2):91–106, 1975.
11. GOODMAN LS, and GILMAN A. The Pharmacological Basis of Therapeutics, 5th ed. New York, MacMillan Publishing Co, Inc, 1975, pp 1285–1288.

FIGURE 2.—a. Typical field of Novikoff hepatoma cells from control animals. The nucleoli are frequently large and irregularly shaped (arrows), although in some cells they are rounded and of intermediate size (pointers) (× 2680). b. Novikoff hepatoma cells after treatment with actinomycin D (1 mg/kg). The No. of aberrant nucleolar forms is greatly increased when compared with the control field. The large nucleolus (arrow) is composed of RNP trabecules and rings. The ring-shaped nucleolus (large pointer) contains a dark "nucleolar cap" (small pointer). Micronucleoli are also present (small arrows). The nucleoli of cells A and B are enlarged in fig 1h (× 2000). c. Novikoff hepatoma cells after treatment with adriamycin (10 mg/kg). In cell A, the large, round, compact nucleolus contains several light areas (small pointers). In cell B, two nucleoli (arrows) consist only of a few RNP trabecules and the other nucleolus is very small (× 2080). d. Novikoff hepatoma cells after treatment with adriamycin (10 mg/kg). 2 small, ring-shaped nucleoli are shown by arrows. In the lower left cell, a large nucleolus is segmented or undergoing fragmentation (pointers) (× 2040).

Murine Myeloid Leukemia: I. Pathophysiology and Drug Sensitivity [1,2]

Harvey D. Preisler,* Sigurdur Bjornsson, and Mayumi Mori [3,4]

SUMMARY

The pathophysiology of serially passaged myeloid leukemia of the RFM mouse was studied. The disease was characterized by progressive splenomegaly and infiltration of both marrow and spleen by myeloblasts. The animals became anemic and there was an associated erythroid hyperplasia in the spleen. Leukemic spleen cells obtained from animals early in the course of the leukemia were less malignant than those obtained from preterminal mice. The leukemia is most sensitive to alkylating agents but is also responsive to antimetabolites.

[Cancer Treat Rep 61:1259–1268, 1977]

Virtually all murine leukemias used as models for the study of acute leukemia in man are lymphoid leukemias (1). Although these systems have provided much useful information about leukemia in general, they are not good models for acute myelocytic leukemia (AML), the most common leukemia found in adults. We have begun to explore the use of murine myeloid leukemia (MML) in the RFM mouse as a possible model for human AML. This paper will present our initial findings with regard to the pathophysiology and chemotherapy of this disease.

METHODS

Two- to 5-month-old RFM/UN mice bred by brother-sister matings in our animal facility were used throughout these studies. Eight mice were housed to a cage and were given food and water ad libitum.

RFM/UN mice are an inbred strain which were kindly supplied by Dr. John Storer of the Oak Ridge National Laboratories. The myeloid leukemia cells used to establish the leukemia in these mice were also supplied by Dr. Storer. The original donor of these cells was an irradiated RFM/UN mouse who developed leukemia in 1964. The mouse received 300 rads of whole body irradiation at 3 months of age and was found to be leukemic 9 months later. After eight in vivo passages the leukemic cells were frozen in 1965. They were subsequently thawed, re-passaged seven times, and re-frozen in 1967. The cells were sent to us in a frozen state in June 1975. They were thawed at that time and have been in continuous passage to date. The cells used in these studies were in their 26th to 40th passage.

Maintenance and Passage of the Leukemia

To passage the leukemia, a spleen obtained from an animal with advanced disease was placed into cold isotonic saline and forced through a sterile stainless steel mesh. The resultant cell suspension was repeatedly aspirated through a 27-gauge needle to disaggregate any clumps. The number of cells which excluded trypan blue was determined (> 99%) and an appropriate number of cells in 0.2 ml of isotonic saline were injected iv into a lateral tail vein. For routine passage, 1×10^6 spleen cells from animals whose spleens were ≥ 500 mg were inoculated into normal mice every 7–10 days. In the experiments described below, the cells were pre-

[1]Received Aug 4, 1976; revised Apr 7, 1977; accepted Apr 13, 1977.

[2]Supported by Public Health Service grant CA-17785 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3]Department of Medicine A, Roswell Park Memorial Institute, Buffalo, NY.

[4]We would like to thank G. Christoff, B. Bryant, S. Alamru, S. Gottlieb, C. Shields, and E. Taylor for their excellent technical assistance. We would especially like to thank Dr. Maurice Barcos for his advice and assistance in studying the pathology of this disease and C. Scharlau for preparing the figures which appear in this paper.

*Reprint requests to: Dr. H. D. Preisler, Department of Medicine A, Roswell Park Memorial Institute, 666 Elm St, Buffalo, NY 14263.

pared in an identical manner, diluted, and inoculated by the route described.

Histochemical and Pathologic Studies

Autopsies were performed on every mouse that died during these experiments. The abdominal, thoracic, and cranial cavities were opened and inspected. The liver, kidney, and spleen were removed and weighed. When appropriate, portions of the spleen, liver, lymph nodes, femur, heart, lungs, thymus, brain, and subcutaneous tumors were removed, placed in Zenker's fixative, sectioned, and stained with PAS or hematoxylin and eosin.

Single-cell suspensions of bone marrow and spleen cells were prepared as previously described (2). Slides were made using a cytocentrifuge and stained for peroxidase, chloroacetate esterase, sudan black, nonspecific esterase (NSE), glycogen (PAS), and leukocyte alkaline phosphatase (LAP).

Studies on the Course of the Leukemia

In experiments 1 and 2, mice were injected with 1×10^6 leukemic spleen cells obtained from a mouse with preterminal (the day the animals begin to die) leukemia. On each day thereafter, three mice were anesthetized and peripheral blood for counts and smears was obtained by axillary artery and vein incision. In experiment 2, hematocrit levels and reticulocyte counts were also determined. After bleeding, the mice were killed, their internal organs examined, and their spleens removed and weighed. Splenic cells for morphologic examination and for malignancy assay were obtained as described above. Bone marrow specimens were obtained each day for total cell and differential counts. A single observer in a single-blinded fashion scored 300 cells for differential counting.

In experiment 2, appropriate mice were hypertransfused with blood obtained from syngeneic mice as previously described (3). The leukemic cells were transplanted iv into these mice immediately after the second hypertransfusion.

Malignant Potential Assays

In both experiments 1 and 2, leukemic spleen cells were harvested as described above from three mice per day (Days 4, 5, 7, and 8 in experiment 1 and Days 4, 5, 8, and 9 in experiment 2). The malignant potential of the leukemic spleen cells was assayed by pooling the three individual spleen cell suspensions and inoculating 1×10^6 cells and serial log dilutions of the pooled spleen cell suspension (10^5, 10^4, 10^3, and 10^2 cells) iv into syngeneic mice (eight mice per cell dilution). These recipient mice were followed until death at which time an autopsy was performed and the spleens were weighed.

Chemotherapy

Animals were inoculated iv with 1×10^6 spleen cells from a preterminal mouse. The mice were palpated each day and the administration of chemotherapeutic agents was initiated at the time of the appearance of detectable splenomegaly (estimated spleen weight, 250–300 mg). The animals were randomly assigned to treatment groups and treatment was started simultaneously in all groups.

The drugs were dissolved in phosphate buffered saline (PBS) and all were administered ip in a final volume of 0.01 ml/g of mouse weight, except daunorubicin which was administered iv. Control mice received PBS only in the same volume. Mice were treated according to a predetermined schedule. When treatment prolonged survival, the therapy was continued for twice the duration of survival of the control mice from the start of PBS injections. There were ten mice in each treatment group and each experiment was repeated three times. All animals were autopsied.

Survival of the treated mice was measured as the percentage increase in median survival time (MST) compared to untreated control mice from the start of therapy.

RESULTS

Characterization of MML

Mice inoculated iv with 1×10^6 spleen cells obtained from a preterminal leukemic mouse all died within 2 days of each other 7–10 days after inoculation (vide infra). These animals appeared healthy until 24 hours before their death when their fur became roughened and the animals became hunched up. Gross pathologic examination revealed splenomegaly (600–800 mg) and lymphadenopathy. Hemorrhage was not present. Microscopic examination revealed the following: (*a*) There was infiltration of the spleen by primitive granulocytic cells ranging from blast cells to myelocytes with an accompanying erythroid hyperplasia and an increase in megakaryocytes (fig 1A). (*b*) The bone marrow was packed with myeloid elements. (*c*) There was leukemic cell infiltration of the liver and the lymph nodes (in some mice the infiltration of the liver was so severe that the structure of this organ was disrupted) (*d*). The thymus, kidneys, lungs, heart, brain, and meninges appeared normal.

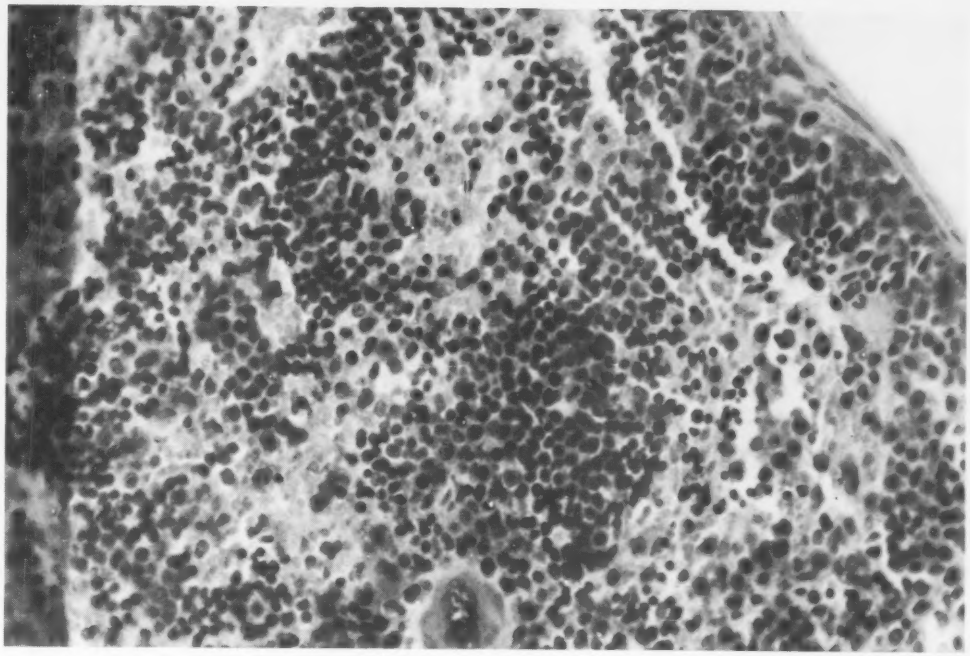


FIGURE 1.—Morphology of MML. (A) Histologic section of spleen from leukemic mouse. PAS stain (× 100). Dark nuclei are those of erythroblasts. Lighter areas are infiltrations of primitive myeloid cells. At the extreme right of the figure are several mature granulocytes and a metamyelocyte with a "doughnut-shaped" nucleus. A megakaryocyte is evident at the bottom of the figure. (B) Wright-Giemsa stain of cytocentrifuge preparation of myeloblasts obtained from the bone marrow of a leukemic mouse (× 1000).

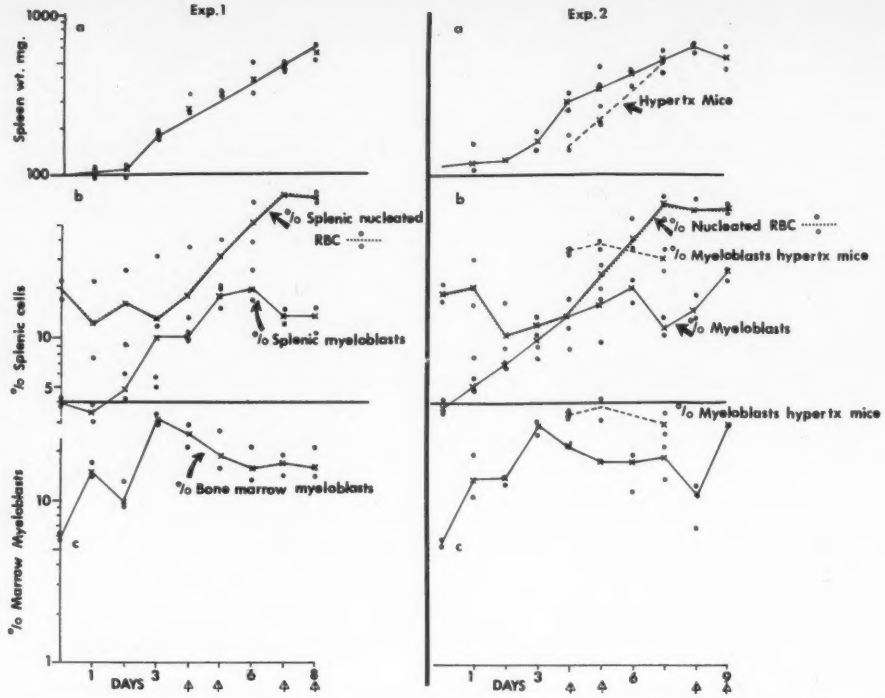


FIGURE 2.—Kinetics of MML in experiments 1 and 2. Individual measurements are given by either open or closed circles. Means are indicated by ×. Broken lines refer to measurements of hypertransfused mice. (a) Increase in spleen weight during course of the disease. (b) Change in proportion of splenic myeloblasts and nucleated erythrocytes during course of the disease. Open circles refer to measurements of erythroid precursors and closed circles refer to measurements of myeloblasts. (c) Change in proportion of bone marrow myeloblasts during course of disease. Arrows on abscissa indicate the days in each experiment in which spleen cells were removed for assessment of malignant potential (figs 3 and 4).

Some mice had thrombi present in pulmonary blood vessels. The thrombi consisted of leukemic cells alone or a mixture of fibrin and leukemic cells.

Staining Characteristics of the Leukemic Spleen and Bone Marrow Cells

The leukemia was characterized by an increase in the proportion of myeloblasts (fig 1B). A small proportion of the leukemic cells appeared to be capable of maturing to the myelocyte and metamyelocyte level. The leukemic cells possessed peroxidase and chloroacetate esterase activity and were sudan black positive. The leukemic cells were negative on staining for PAS, NSE, and LAP. This staining pattern is characteristic of immature cells of the granulocytic series (4).

Route of Inoculation of Leukemic Spleen Cells

Ip inoculation of leukemic spleen cells resulted in a disease which was pathologically indistinguishable from iv inoculation of cells. The animals died with splenomegaly and without ascites. Death resulting from ip inoculation appeared to occur 1–2 weeks later than death due to a comparable iv inoculation of leukemic cells.

Three mice were inoculated sc in the inguinal region with 1×10^6 leukemic spleen cells and three mice received identical inoculations sc in the dorsal-lumbar region. The latter group of mice died 17 days later with spleen weights of 670, 640, and 570 mg. There was no evidence of subcutaneous tumor formation. The former group of mice died 16, 16, and 19 days after inoculation with spleens weighing 400, 500, and 430 mg. These mice developed tumors at the site of inoculation. At the time of death the tumors weighed 100, 70, and 60 mg respectively. On pathologic examination the tumors consisted primarily of primitive granulocytic cells but there were areas containing cells which appeared to be normoblasts and megakaryocytes.

Course of MML

Animals that received 1×10^6 spleen cells from leukemic mice were killed each day. All of the mice that were not killed died on Day 8 in experiment 1 and on Day 9 in experiment 2. Between the second and third day after transplantation the spleen weight began to increase. The rate of increase in spleen weight appeared to be exponential for several days and then began to increase within 48 hours of transplantation. The increase approximated an exponential function until Day 6 when 20% of the cells were myeloblasts. The proportion of myeloblasts declined to 12%–14% on Days 7 and 8 (fig 2b). Although the proportion of myeloblasts in the spleen declined, the absolute number of myeloblasts (as approximated by multiplying percent myeloblasts × spleen weight) increased throughout the course of the disease as a result of the progressive increase in spleen weight.

Concomitant with the progressive splenomegaly was a significant increase in both the proportion and the number of nucleated erythroid precursors in the spleen (fig 2b). This increase was first noted 5 days after transplantation and thereafter paralleled the progressive increase in the absolute number of myeloblasts. The proportion of lymphocytes in the spleen fell from 70%–80% on Day 0 to 10%–20% on Day 8 or 9. The proportion of mature granulocytes and plasma cells did not change during the course of the disease. It is of interest that there was an apparent fall in the proportion of splenic erythroblasts during the initial 3–4 days after inoculation of leukemic spleen cells.

The number of femoral marrow cells did not change significantly during the course of the disease. It varied from 10 to 13 × 10^6. The proportion of myeloblasts in the femur increased from 5.6% at 0 time to 30% on Day 3 and then gradually declined (fig 2c). The variability in the proportion of nucleated erythroid cells in the bone marrow was so great that no conclusions could be drawn.

Wbc counts remained in the 8000–9000 range until the day of death at which time they rose to 25,000 ± 9400 in experiment 1 and to 37,800 ± 5900 in experiment 2. Circulating leukemic cells were not detected until the day of death. In experiment 2 the blood hematocrit levels and reticulocyte counts were measured on Days 3, 5, 6, and 7. On Day 2 the hematocrit levels were 44 ± 0.7 and the reticulocyte counts were 0.93 ± 0.2, which were unchanged from control values (45 ± 0.9 and 1.6 ± 0.7). On Day 5 the average hematocrit level was 47 ± 2 with an apparent elevation in the reticulocyte count to 3.6 ± 0.4. On Days 6 and 7 the average hematocrit levels were 27 ± 0.6 and 28 ± 1.2 while the average reticulocyte counts were 4.6 ± 0.8 and 6.6 ± 1.3 respectively. Hence, the disease appeared to be accompanied by a progressive anemia and reticulocytosis.

Hypertransfusion Study

In experiment 2 a subgroup of mice were hypertransfused with packed erythrocytes prior to being inoculated with leukemic spleen cells. They were killed on Days 4, 5, and 7. On each day of death their average spleen weights were less than that of the nonhypertransfused leukemic mice (fig 2a). Hypertransfusion was effective in suppressing erythropoiesis since the percent of erythroblasts in the spleen on these days was reduced to 5%, 6.5%, and 4% respectively (compared to 15%, 32%, and 70% in nonhypertransfused leukemic mice on the same day). The percentage of splenic myeloblasts was greater in the hypertransfused mice (fig 2b) and on Day 7 the product of percent myeloblasts × spleen weight was also greater. Similar observations were made on the bone marrows of these mice. On Days 4, 5, and 7 the percent of nucleated erythroblasts in the bone marrow was 0.6%, 1.5%, and 0.7% respectively (compared to 6.5%, 15%, and 16% for the corresponding nonhypertransfused leukemic mice). As in the spleen, the proportion of myeloblasts was greater in the marrows of hypertransfused mice than in their nonhypertransfused counterparts (fig 2c).

Malignant Potential of Splenic Myeloblasts

The malignant potential of splenic cells was tested at different times during the course of the leukemia. Figure 3 compares the survival curves of mice that received leukemic spleen cells from mice with early disease with survival curves of mice that received leukemic cells from preterminal donors. Figure 4 gives the median survival data of all the mice in experiments 1 and 2. In every case, inoculation of 1×10^6 leukemic spleen cells resulted in the death of the recipient mice. The interval between inoculation of leukemic spleen cells and the median time to death was progressively shortened when animals were inoculated with cells obtained from animals in later stages of the disease. The greater malignant potential of leukemic spleen cells from late disease compared to early disease was also evidenced by the fact that as few as 10^4 leukemic spleen cells derived from mice with late disease were lethal, whereas it took $\geq 10^5$ cells from mice with early disease to kill the recipient.

There was a direct relationship between the duration of survival and the weight of the spleen at

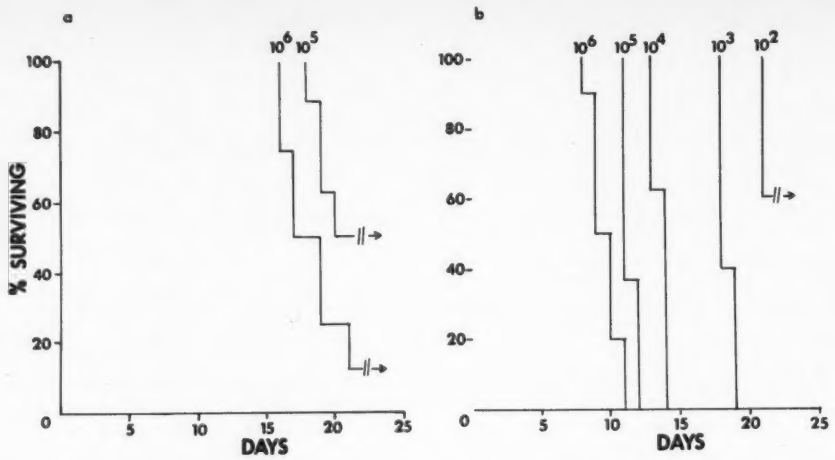


FIGURE 3.—Survival curves of mice used in malignancy assays. Abscissa gives days of survival after transplantation. Cells were harvested in experiment 2, 4 (a) and 9 (b) days after inoculation of mice with leukemic spleen cells. Serial log dilutions ($10^6 \rightarrow 10^2$ cells) were inoculated into recipient mice and the time to death was determined. Broken lines and arrows indicate survival of mice for > 90 days. No mouse died that received ≤ 10^4 spleen cells from a leukemic mouse 4 days after transplantation of the leukemia (a).

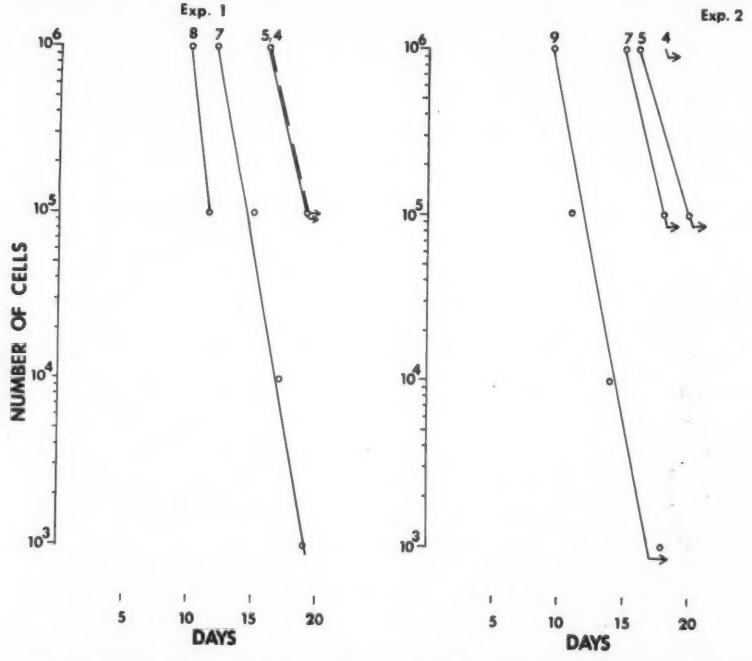


FIGURE 4.—Median survivals of recipients of spleen cells from leukemic mice. Nos. above curves indicate day of harvest of the spleen cells from the donor mouse. Abscissa gives survival (days) after inoculation and ordinate gives No. of cells injected. Arrows indicate long-term survivors (> 90 days). In experiment 1, less than one half of the mice that received 10^4 spleen cells obtained from 4- and 5-day leukemias died. Recipients of Day 8 cells received only 10^6 or 10^5 spleen cells. In experiment 2 the only recipients of Day 4 cells that died were those receiving 10^6 cells. Recipients of Day 5 or 7 cells died only if they were inoculated with ≥ 10^5 cells.

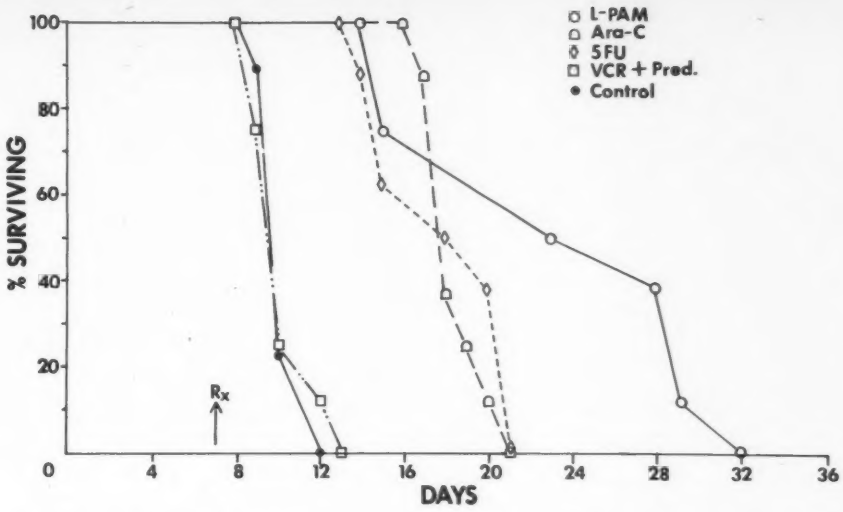


FIGURE 5.—Animals were inoculated with 1×10^6 spleen cells from leukemic mice at 0 time. The arrow (R_x) indicates the appearance of splenomegaly and the initiation of therapy.

death (table 1). This was the case whether one compared the spleen size at death among the different groups that received 10^6 cells or within each group among mice that received different numbers of cells.

Chemotherapy Studies

In these experiments splenomegaly was detected 7 days after transplantation and chemotherapy was initiated at that time. Table 2 reports the effects of the various chemotherapeutic regimens on the survival of the leukemic mice. The alkylating agents cyclophosphamide, melphalan, and BCNU were the most active compounds; they produced at least a fivefold increase in survival. Antimetabolites such as cytosine arabinoside, methotrexate, 6-mercaptopurine, and 5-azacytidine also prolonged survival with twofold to threefold increases. Representative survival curves are illustrated in figure 5. Mice treated with daunorubicin and *N*-methylacetamide died of drug toxicity with normal-sized spleens.

DISCUSSION

The disease produced by transplantation of leukemic spleen cells was characterized by extensive invasion of the spleen, liver, and lymph nodes as has been previously described (5). Wbc and differential counts were within normal limits until 1 or 2 days prior to death. As evidenced by their morphology and cytochemistry, the leukemic cells were myeloblasts, a minority of which could differentiate to at least the metamyelocytic level. The leukemic cells were, as has been reported by others, aneuploid with 39 chromosomes (6). Using the karyotypic abnormality as a marker, we have found that under the influence of the colony-stimulating factor the leukemic cells could differentiate in vitro into macrophages and mature granulocytes.[5]

When sufficient numbers of leukemic spleen cells were inoculated, disseminated malignancy resulted from iv, ip, or sc inoculation. Several observations deserve discussion. Cells inoculated sc in the inguinal region appeared to be more likely to produce subcutaneous tumors than cells inoculated in the dorsal-lumbar region. In one of three subcutaneous tumors, there was evidence of erythropoiesis. It is not known if the erythropoietic activity resulted from the proliferation of normal splenic elements which were inoculated sc along with the leukemic cells or if they represented erythroid differentiation by the leukemic cells. The karyotypic studies referred to below suggest that the former is the case. The rapidity of development of the leukemia produced by transplantation of the spleen cells and the length of time required to produce leukemia with cell-free filtrates (7) demonstrated that the disease resulted from the proliferation of the transplanted cells themselves and not from virus that the cells produce.

[5]Mori M, Preisler HD, Oshimura M, et al. Manuscript in preparation.

TABLE 1.—Relationship between survival of mice and spleen weight

Experiment No.	Day cells obtained from donor mouse	No. of cells inoculated	Median survival of recipient mice (days)	Median spleen weight (mg)	Mean spleen weight (mg) ± SE
1	4	10^6	17	760	760 ± 40
	5	10^6	16	810	780 ± 20
	7	10^6	12	730	740 ± 60
	8	10^6	10	640	640 ± 20
1	4	10^5	20	920	800 ± 45
	5	10^5	19	1100	880 ± 60
	7	10^5	15	820	780 ± 30
	8	10^5	11	720	710 ± 40
2	4	10^6	17	850	811 ± 85
	5	10^6	16	850	823 ± 41
	8	10^6	15	790	761 ± 36
	9	10^6	9.5	550	546 ± 23
1	7	10^4	17	980	890 ± 35
	7	10^5	15	820	780 ± 30
	7	10^6	12	730	740 ± 60
2	9	10^3	18	740	838 ± 91
	9	10^4	14	711	711 ± 44
	9	10^5	11	600	626 ± 30
	9	10^6	9.5	550	546 ± 23

TABLE 2.—Effect of chemotherapy

Treatment	Dose (mg/kg)	Schedule*	MST†	% increase in lifespan	Spleen weight (mg)‡ Median	Mean
Control	—	—	3	—	501	500
Cyclophosphamide	200	q10d	21	600	710	677
Melphalan	7.7	q4d	18.5	517	198	160
BCNU	30	q7d	18	500	250	218
Cytosine arabinoside	100	qd	11	267	303	280
5-Fluorouracil	60	q4d	12.5	316	323	310
Methotrexate	15	q2d	11	266	579	665
6-Mercaptopurine	80	qd	7	133	305	334
5-Azacytidine	30	q2d	5	67	274	250
Daunorubicin	12	qd × 3	7	133	93	90
Methyl-GAG	65	qd	7.5	150	270	230
N-methylacetamide	15%§	qd	9	200	125	141
Vincristine + Prednisone	0.5 30	Day 1 qd	3	0	450	330

*Chemotherapy administered for twice the survival of controls from initiation of therapy. For example, animals that received cytosine arabinoside qd received 6 inoculations (1/day) since controls survived 3 days. Animals that received cyclophosphamide or BCNU received only a single inoculation. Animals receiving qod therapy received 3 doses of drug. Animals receiving melphalan or 5-fluorouracil received 2 inoculations.

†Median survival time. Days from initiation of treatment (Day 7 after tumor implantation).

‡Spleen from healthy nonleukemic mouse = 100–150 mg.

§0.01 ml/g mouse weight of 15% (vol/vol) *N*-methylacetamide.

An intense erythroid hyperplasia accompanied this disease. This hyperplasia accounted for the observation that the relative proportion of myeloblasts decreased during the disease despite the fact that the absolute number increased. The hypertransfusion studies demonstrated that the proliferating erythroid precursors responded to normal control mechanisms suggesting that the erythroid hyperplasia (and the accompanying increase in splenic megakaryocytes) was reactive in nature and not a result of the erythroid differentiation of leukemic stem cells. Furthermore, preliminary karyotypic studies using hypertransfused mice suggested that the erythroid precursors possessed 40 chromosomes rather than 39 as did leukemic myeloblasts. A similar splenic erythroid hyperplasia has been reported in rats bearing transplanted MML (8,9).

The difference in malignant potential between cells harvested early in the course of disease and those harvested late was an unexpected observation. This difference was reflected in two ways: (1) 1×10^6 cells obtained from animals with preterminal disease killed mice after a shorter interval than did the same number of cells obtained from animals which were earlier in the course of the disease, and (2) cells from preterminal mice were several logs more potent in producing leukemia than were cells obtained earlier in the course of the disease. This latter observation clearly establishes the fact that the small differences in the absolute number of myeloblasts that were inoculated (percent myeloblasts × number of cells injected) into the recipient mice used for assays of malignant potential could not possibly account for the observed differences in malignancy. An explanation for this observation would also have to include the observation that the cells which were routinely passed from week to week had the same potential as those studied in this experiment, ie, that the cells themselves appeared to vary and not the host. Expressed differently, the same cells appeared to vary in malignant potential with the independent variable being the time in the disease when the cells were removed from the leukemic spleen for assessment of malignant potential.

While there is as yet no explanation for these observations, perhaps the proliferative state of the leukemic cells at the time they were removed from the diseased mice was a factor in determining the malignant potential of the cells. If the proliferating cells were more sensitive both to removal and the in vitro conditions than were nonproliferative cells, then although the total number of injected cells was identical the number of viable cells might not be. We estimated the number of leukemic cells on the basis of the percent of myeloblasts present in the cell suspension. Perhaps early in the disease the majority of the myeloblasts were normal precursors which were morphologically indistinguishable from the malignant cells. Hence, in early disease we could have been inoculating far fewer leukemic cells than we thought. Our observations on the erythroid and megakaryocytic hyperplasia in the spleens of these mice make this explanation a reasonable one. We are currently exploring this possibility with karyotypic studies. Perhaps the immunogenicity of the cells vary depending upon the stage of the disease, with the cells from animals with early disease being more immunogenic. Alternatively, since in these studies nonleukemic spleen cells were inoculated with the leukemic spleen cells, it is possible that in early disease these normal cells were immunocompetent and retarded the growth of the leukemic cells while similar cells from mice with late disease may no longer be immunocompetent. To further investigate this possibility, studies are currently under way using purified populations of myeloblasts.

A variety of chemotherapeutic agents are effective in treating this MML. While optimal treatment schedules have not as yet been determined, the alkylating agents appear to be most effective. In fact, previous studies demonstrated differential sensitivity between leukemic and normal spleen colony-forming units to cyclophosphamide (10).

Of the drugs tested to date, all but four which have activity in this system have been shown to be active, either alone or in combination, in the treatment of AML in man. Methotrexate, the fourth drug, has little effect against human AML. Of particular interest was the observation that *N*-methylacetamide, one of a series of compounds which induce the differentiation of Friend leukemia cells in vitro (11) and alter their growth in vivo (12), also prolonged the survival of mice with MML.

In conclusion, the leukemia under discussion is clearly myeloid in nature and its growth kinetics are sufficiently reproducible as to permit the study of MML in a small, easy-to-manage mammal.

LIST OF COMPOUNDS

5-Azacytidine: NSC-102816; CAS reg. No. 320-67-2; *s*-triazin-2(1*H*)-one, 4-amino-1-β-D-ribofuranosyl-

BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate

Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride

Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6

5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8

Melphalan: NSC-8806; CAS reg. No. 3223-07-2; alanine, 3-[*p*-[bis(2-chloroethyl)amino]phenyl]-, monohydrochloride, L-

Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-

Methyl-GAG: NSC-32946; CAS reg. No. 24968-67-0; guanidine, 1,1'-[(methylethanediylidene)dinitrilo]di-, dihydrochloride, monohydrate

6-Mercaptopurine: NSC-755; CAS reg. No. 6112-76-1; purine-6-thiol, monohydrate

***N*-methylacetamide:** NSC-747; CAS reg. No. 79-16-3

Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

REFERENCES

1. FREI E, SCHABEL FM, and GOLDIN A. Comparative chemotherapy of AKR lymphoma and human hematological neoplasia. Cancer Res 34:184, 1974.
2. PREISLER HD, and HENDERSON ES. The effect of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and cytosine arabinoside (ara-C) on hematopoiesis in the mouse. J Natl Cancer Inst 47:971, 1971.
3. ———. Effect of suppression of erythropoiesis on hematopoietic stem cells in the mouse. J Cell Physiol 79:103, 1972.
4. GUNZ F, and BAIKIE A, eds. The pathology and pathophysiology of the leukemic cell. *In* Leukemia. New York, Grune and Stratton, 1974, pp 133–163.
5. PARSONS DF, UPTON AC, BENDER MA, ET AL. Electron microscope observations on primary and serially passaged radiation-induced myeloid leukemia in the RF mouse. Cancer Res 22:728, 1962.
6. HUSSEINI S, FREID W, KNOSPE WH, ET AL. Dynamics of leukemia and normal stem cells in leukemic RFM mice. Cancer Res 36:1784, 1976.
7. JENKINS VK, and UPTON AC. Cell free transmission of radiogenic myeloid leukemia in the mouse. Cancer Res 23:1748, 1963.
8. HANDLER EE, HANDLER ES, and SCHAEFER AE. Alterations in marrow and splenic hematopoiesis in acute myelogenous leukemia. J Reticuloendothel Soc 5:445, 1968.
9. HANDLER EE, and HANDLER ES. Erythropoiesis in rats with acute myelogenous leukemia. J Reticuloendothel Soc 7:238, 1970.
10. TANAKA T, CRAIG AW, and LAJTHA LG. A kinetic study on murine myeloid leukemia. Br J Cancer 24:138–147, 1970.
11. PREISLER HD, and LYMAN G. Differentiation of erythroleukemic cells in vitro: properties of chemical inducers. Cell Differ 4:179–185, 1975.
12. PREISLER HD, BJORNSSON S, MORI M, ET AL. Inducers of erythroleukemic cell differentiation in vitro: effects of in vivo administration. Br J Cancer, 1976. In press.

Distribution and Metabolism of Vincristine in Mice, Rats, Dogs, and Monkeys[1,2,3]

Salah M. El Dareer,[4,*] Vivian M. White,[4] Francis P. Chen,[4] L. Bruce Mellett,[4,5] and Donald L. Hill[4]

SUMMARY

An extraction procedure that allows measurement of [^{3}H]vincristine and its metabolites in biologic fluids and tissues has been developed and used to determine the distribution and metabolism of vincristine in mice, rats, dogs, and monkeys. Very little tritium exchange occurred during the experiments. In mice and rats injected ip with [^{3}H]vincristine, the peak serum levels of drug were reached at 15 minutes. For both mice and rats, the concentration of vincristine metabolites rose in the serum for 30 minutes, then remained high through 3 hours. For dogs and monkeys given an iv dose of [^{3}H]vincristine, the initial serum levels fell rapidly, with half-lives for the first phase of 6 and 10 minutes respectively. The half-life for both of the second phases was 190 minutes. For both species, the serum level of metabolites fell for 2 hours, then remained relatively constant. These results were not appreciably altered when the animals were injected simultaneously with prednisone. Compared to serum levels in mice, rats, dogs, and monkeys, there was a marked accumulation of [^{3}H]vincristine in all tissues examined, except the brain. The pancreas, spleen, kidneys, lungs, and liver of dogs and monkeys contained the largest amounts. The tissue distribution was not greatly different in dogs and monkeys injected simultaneously with prednisone. Urinary excretion of intact vincristine and its metabolites was observed for all four species. Both vincristine and its metabolites appeared in the bile of dogs and monkeys.

[Cancer Treat Rep 61:1269–1277, 1977]

Vincristine, one of the *Vinca rosea* alkaloids, is active against a variety of experimental tumors and human cancers (1,2). As a single agent, it is particularly effective against acute leukemia, Wilms' tumor, rhabdomyosarcoma, and various lymphomas. When combined with prednisone,[6] vincristine induces complete responses in about 90% of patients with acute lymphoblastic leukemia (3). Vincristine, especially in combination with other agents, is also effective against several other types of cancer. By a mechanism not completely defined, vincristine inhibits the cell cycle at metaphase (3). The inhibition is apparently accomplished after the agent binds to tubulin, a component of cellular microtubules (4).

In this paper, we report the development of a procedure for measuring [^{3}H]vincristine and its metabolites in biologic samples and the utilization of this method to determine the serum and tissue levels of these compounds in mice, rats, dogs, and monkeys given [^{3}H]vincristine. Preliminary reports of portions of this work have been presented (5,6).

MATERIALS AND METHODS

Bio-Solv BBS-3 solubilizer was purchased from Beckman Instruments, Palo Alto, Calif, and Aquasol and Liquifluor scintillation solutions from the

[1]Received Sept 27, 1976; revised Mar 21, 1977; accepted Apr 20, 1977.

[2]Supported by contract N01-CM-43780 from the Division of Cancer Treatment (DCT), National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3]Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1). Supplied by the Developmental Therapeutics Program, DCT, NCI.

[4]Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Ala.

[5]*Present address:* USV Pharmaceutical Corp, Tuckahoe, NY.

[6]Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-.

**Reprint requests to:* Salah M. El Dareer, DVM, Kettering-Meyer Laboratory, Southern Research Institute, 2000 9th Ave S, Birmingham, Ala 35205.

New England Nuclear Corp, Boston, Mass. All radioactive samples were counted in a liquid scintillation spectrometer equipped with an external standard. Thin-layer sheets of silica gel (type SA) were obtained from the Gelman Instrument Co, Ann Arbor, Mich. [^{3}H]vincristine sulfate was supplied by the Division of Cancer Treatment, National Cancer Institute.

[^{3}H]vincristine sulfate, recrystallized from benzene, had a constant specific activity of 42 millicuries/mmol and a radiopurity of 95%–100%. Prior to injection, the [^{3}H]vincristine was diluted with unlabeled drug to a specific activity of 8.8 millicuries/mmol. Solutions for injection were prepared in 0.9% sodium chloride. Purity of the compound was established by paper chromatography in nine different solvent systems and thin-layer chromatography on silica gel sheets in six different solvent systems. Radioactivity on the developed strips and sheets was measured by cutting 0.5-cm strips, placing them in vials containing 1 ml of water, and subjecting the vials, with Aquasol solution added, to scintillation counting. Standard unlabeled vincristine sulfate was detected under ultraviolet light or by spraying with iodoplatinic acid reagent (7).

Animals used in this study were male BDF_1 mice weighing 20–30 g, male Sprague-Dawley rats weighing 200–280 g, female beagle dogs weighing 7.8–10.2 kg, and female rhesus monkeys weighing 3.3–6.7 kg. All animals were starved overnight prior to use. Urine was collected by catheters. Blood was collected from the axillary region of mice rendered unconscious with carbon dioxide shortly before collection and from the cephalic or saphenous veins of monkeys and dogs. Bile was collected in a syringe by aspiration from the gall bladder. Various organs were collected, blotted on filter paper, placed on solid carbon dioxide, and homogenized, after thawing, in 4 volumes of ethyl alcohol with a Brinkman Polytron equipped with a P-10 generating head. At the time of collection, contents of the intestines were removed. Pooled serum and tissues from five mice or three rats, and individual serum (one to five dogs and one to seven monkeys per time period) and tissue (one animal per time period) samples from dogs and monkeys were used for each determination of vincristine and its metabolites. For determination of total radioactivity in biologic samples, 15 ml of Liquifluor containing Bio-Solv BBS-3 (14:1, vol/vol) was added to a 0.1-ml portion prior to scintillation counting. All assays for radioactivity were performed twice.

[^{3}H]vincristine was extracted from biologic samples (1 ml) after addition of 1 mg of unlabeled vincristine; 3 ml of 1 M phosphate buffer, pH 9.0; and 6 ml of benzene. The preparations were shaken for 40 minutes, then centrifuged to separate the two layers. A 2-ml portion of the benzene layer was transferred to a vial containing Liquifluor scintillation solution, and the radioactivity present was determined. The material remaining in aqueous solution, considered to be metabolites, was counted in Aquasol scintillation solution. Thin-layer chromatography of biologic samples on silica gel sheets in a solvent of chloroform:methanol (1:1, vol/vol) revealed that vincristine had an R_f of 0.80. Metabolites migrated with R_f values of 0.07 (minor) and 0.60 (major). In the benzene extract, only vincristine was present.

RESULTS

Stability, Binding, and In Vitro Metabolism of [^{3}H]Vincristine

A stock solution containing 0.53 mg/ml of [^{3}H]vincristine was prepared and kept at 4°C. Three diluted solutions of 0.27–10.6 μg/ml were prepared in water, serum, homogenized rat brain and liver, and urine. With the benzene extraction procedure, recovery of vincristine added to the samples was 99% ± 4%. For solutions of 53 μg/ml of [^{3}H]vincristine in water and serum, there was no detectable breakdown to other products after incubation at 37°C for 24 hours. However, at 100°C in water, 65% of the [^{3}H]vincristine was destroyed in 1 hour. After incubation of an aqueous solution for 24 hours at 37°C, 99% of the radioactivity could be extracted in benzene, but after 20 days at this temperature, only 66% could be extracted.

When solutions of 100 μg/ml of [^{3}H]vincristine in dog, monkey, or mouse serum were filtered through cellulose dialysis tubing, only 40% of the radioactivity was recovered in the effluent. However, the same recovery was attained when water replaced the serum. These results indicate that vincristine binds only slightly, if at all, to serum proteins but that it binds readily to the dialysis tubing.

Experiments involving incubation for 3 hours of solutions of [^{3}H]vincristine in tissue homogenates of mouse liver, kidneys, spleen, or lungs (prepared in 4 volumes of 0.9% NaCl) and in mouse serum showed that serum did not metabolize vincristine but that liver, kidneys, spleen, and lungs converted 7%–10% of the substrate to metabolites, primarily that with an R_f value of 0.60.

[^{3}H]Vincristine in Mice and Rats

Serum levels of vincristine and its metabolites in mice injected ip with 3 mg/kg are displayed in figure 1. Peak plasma levels of vincristine (0.77 μg/

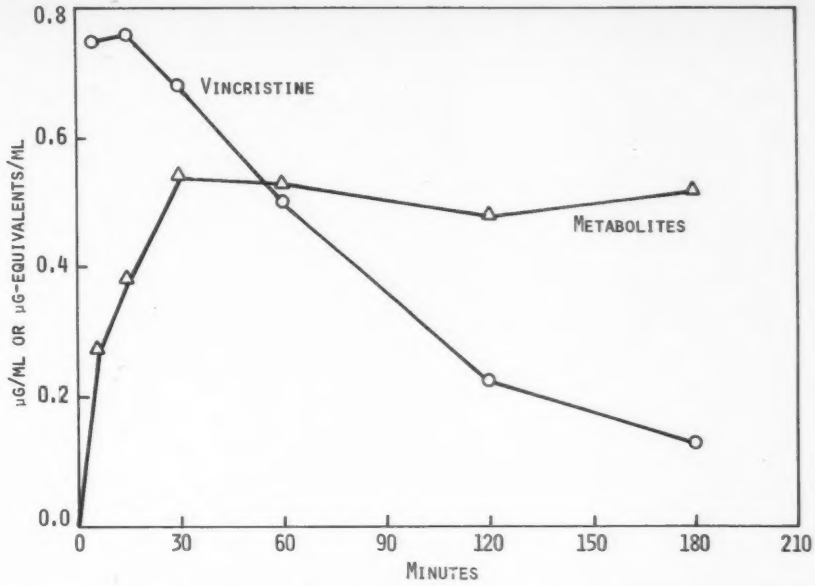


FIGURE 1.—Levels of vincristine and its metabolites in the serum of mice given an ip dose of 3 mg/ kg.

ml) were attained at 15 minutes. Vincristine then disappeared with an apparent half-life of 60 minutes (not shown). Metabolite levels rose for 30 minutes and then remained relatively constant through 180 minutes. Levels of vincristine and its metabolites in the liver, kidneys, spleen, and brain of these mice are shown in figure 2. There is a marked accumulation of unchanged drug in liver, kidneys, and spleen compared to serum levels. However, brain levels of the drug were much lower than serum levels. Metabolite levels were lower in the liver, kidneys, and spleen, but higher than unchanged drug in the spleen and, especially, in the brain.

Only 15% of a 3-mg/kg dose of vincristine was excreted in the urine of five mice in a 6-hour collection period. Half of this was unchanged vincristine and half was metabolites. In a 48-hour collection period, 22% of the total dose was excreted in the urine as vincristine and 25% as metabolites; in the feces, 18% of the total dose was vincristine and 19% was metabolites. At 48 hours, the carcass contained 8% of the total dose as vincristine and 9% as metabolites; the total recovery was 101%. When the urine from these animals was vacuum distilled at 40°C, only 4% of the radioactivity present was recovered in the water.

Serum levels of vincristine and its metabolites in rats given an ip dose of 2 mg/kg of vincristine are shown in figure 3. A peak serum level of 0.33 μg/ml was observed at 15 minutes after drug administration. After this time, the serum level declined with an apparent half-life of 100 minutes. Tissue distribution studies in rats (fig 4) show that vincristine accumulated to a maximum amount in the liver, kidneys, and spleen at 1 hour. The concentration of metabolites, lower than that for the unchanged drug, was also maximum for the kidney at 1 hour. Neither vincristine nor its metabolites were detected in rat brain tissue; the total amount present was < 0.004 μg/g at all times. Of the total radioactivity administered to five rats, 7% was excreted in the urine in 6 hours as vincristine and 7% as metabolites.

[^{3}H]Vincristine in Dogs and Monkeys

Serum levels of vincristine and its metabolites in dogs given iv doses of 0.5 mg/kg are shown in figure 5. At 5 minutes the serum level of vincristine was 0.59 ± 0.08 μg/ml. This amount fell rapidly, with an apparent half-life for the first phase of 6 minutes. Later, there was a slower rate of decline; the apparent half-life of the second phase was 190 minutes. Detectable amounts of vincristine and its metabolites remained in the serum at 8 hours.

The distribution of vincristine and its metabolites in the tissues of individual dogs following a dose of

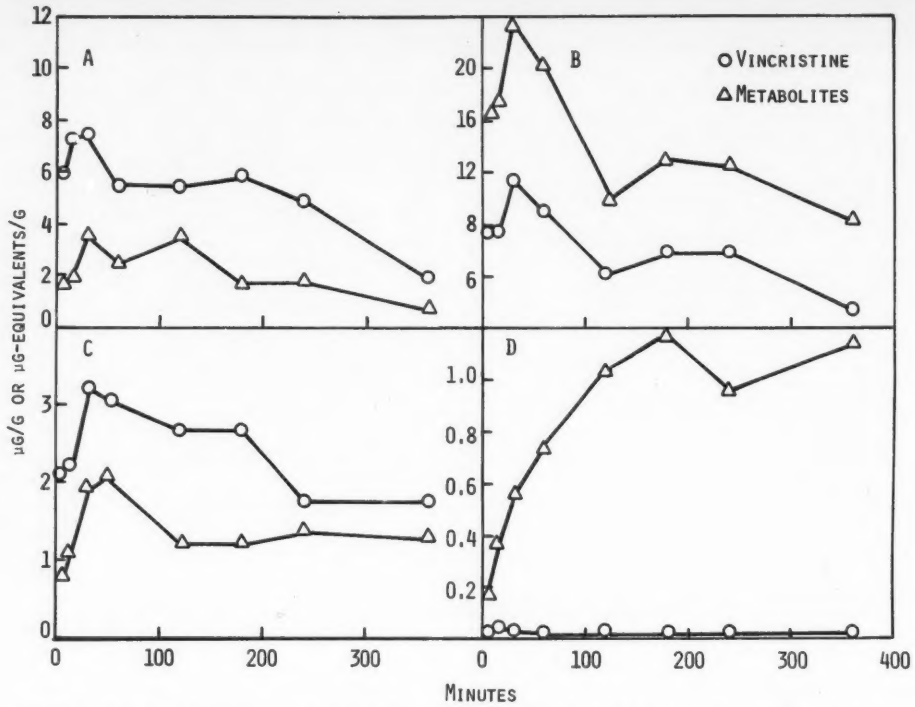


FIGURE 2.—Levels of vincristine and its metabolites in the liver (A), spleen (B), kidneys (C), and brain (D) of mice injected ip with a dose of 3 mg/kg.

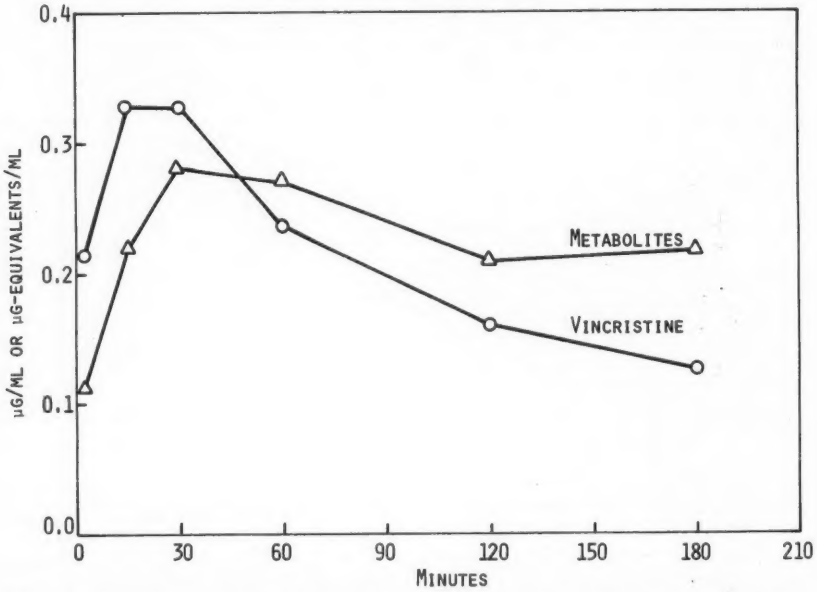


FIGURE 3.—Levels of vincristine and its metabolites in the serum of rats given an ip dose of 2 mg/kg.

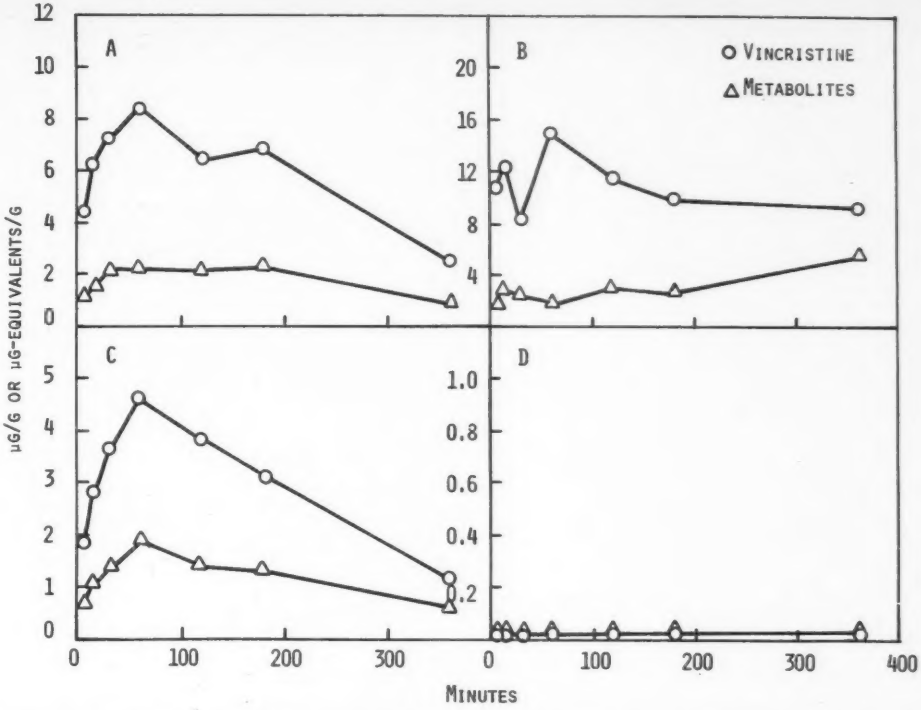


FIGURE 4.—Levels of vincristine and its metabolites in the liver (A), spleen (B), kidneys (C), and brain (D) of rats injected ip with a dose of 2 mg/kg.

0.5 mg/kg is shown in table 1. One dog was killed for each time period. Intact vincristine accumulated in the pancreas, spleen, kidneys, liver, and lungs of dogs to a much greater extent than in plasma. Very little intact vincristine was present in brain tissue. The concentration in the pancreas remained high through 24 hours. (In separate experiments with individual dogs given 1 mg/kg of [^{3}H]vincristine and 2 mg/kg of prednisone simultaneously, the vincristine content of the pancreas was 2.17 μg/g at 48 hours and 1.56 μg/g at 64 hours. At these times, the liver content of vincristine was 0.54 and 0.68 μg/g and the spleen content was 1.35 and 0.88 μg/g respectively.) For most tissues, the concentration of vincristine dropped gradually over an 8-hour period and was greatly reduced at 24 hours. No tissue, except the brain, concentrated the metabolites to a greater extent than intact vincristine.

Figure 6 shows the serum levels of vincristine and its metabolites in monkeys injected iv with 1 mg/kg of [^{3}H]vincristine. At 5 minutes, the serum level of vincristine was 0.77 ± 0.13 μg/ml. As observed for dogs, the initial level fell rapidly. The apparent half-life for the first phase was 10 minutes; that for the second phase was 190 minutes. After 15 minutes, the serum level of metabolites was greater than that for unchanged vincristine.

For both dogs and monkeys given iv doses of [^{3}H]vincristine, the apparent volumes of distribution (60 and 40 liters respectively) were unreasonably high for uniform distribution of the drug.

Table 2 shows the distribution of vincristine and its metabolites in the tissues of individual monkeys. One monkey was killed for each time period. The distribution was very similar to that in dogs, with the liver, kidneys, spleen, and pancreas containing large amounts of vincristine. High concentrations of the agent remained in the pancreas and spleen at 24 hours. Except for the brain, all tissues contained vincristine at higher concentrations than serum; the accumulation in some tissues was 30-fold greater than that in the serum. One notable difference for monkeys was that vincristine metabolites accumulated in most of their tissues to a greater extent than in the tissues of dogs; exceptions were the brain, skeletal muscle, and fat.

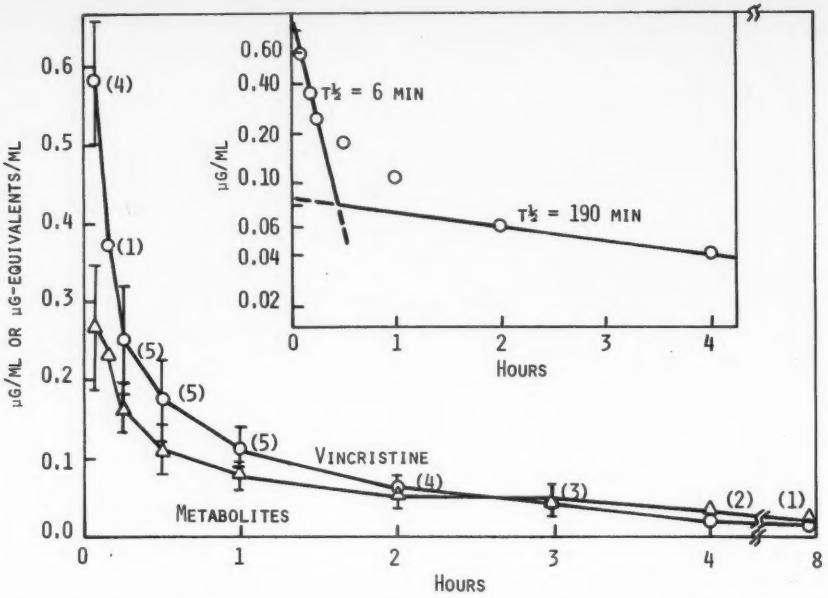


FIGURE 5.—Levels of vincristine and its metabolites in the serum of dogs given an iv injection of 0.5 mg/kg. The Nos. in parentheses indicate the No. of dogs used in determining the values. Standard deviations are indicated for each value. The vertical axis for the inset figure is on a logarithmic scale.

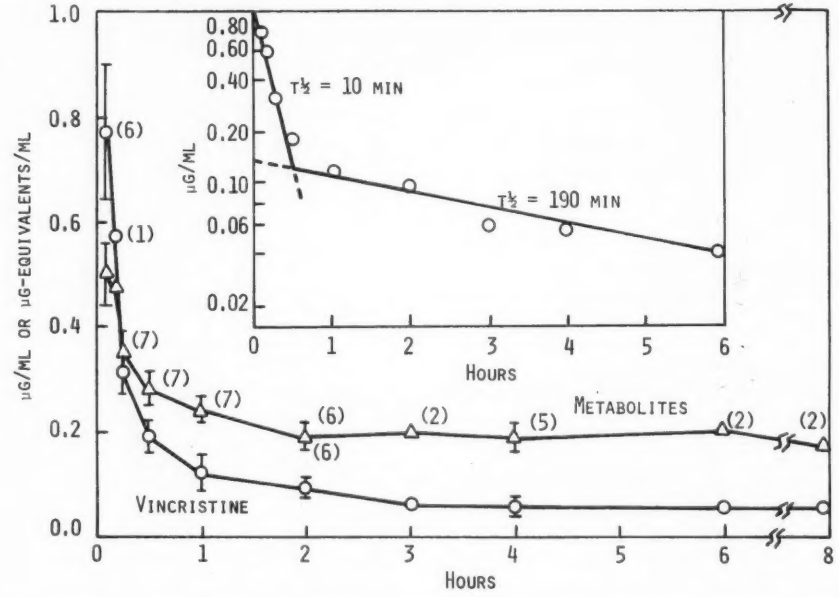


FIGURE 6.—Levels of vincristine and its metabolites in the serum of monkeys given an iv injection of 1 mg/kg. The Nos. in parentheses indicate the No. of monkeys used in determining the values. Standard deviations are indicated for each value. The vertical axis for the inset figure is on a logarithmic scale.

TABLE 1.—Levels of vincristine and metabolites in tissues of dogs given 0.5 mg/kg iv

Tissue	Vincristine (μg/g) Hrs 1	2	4	8	24	Vincristine metabolites (μg equivalents/g) Hrs 1	2	4	8	24
Brain	<0.01	0.01	0.01	0.01	<0.01	0.03	0.01	0.02	0.03	0.04
Liver	1.7	1.5	1.6	1.2	0.49	0.21	0.3	0.27	0.45	0.24
Kidney	2.0	1.5	1.1	0.86	0.26	0.74	0.31	0.24	0.26	0.15
Spleen	2.4	1.8	1.5	1.4	0.61	0.31	0.38	0.24	0.59	0.28
Pancreas	2.4	2.3	2.2	2.7	1.6	0.32	0.44	0.25	0.44	0.22
Bone marrow	0.26	0.23	0.22	0.12	0.11	0.06	<0.01	0.01	<0.01	0.01
Lung	1.7	1.3	1.1	0.94	0.34	0.47	0.43	0.25	0.49	0.09
Heart	1.2	0.96	0.62	0.69	0.25	0.35	0.18	0.23	0.21	0.16
Muscle (gastrocnemius)	0.31	0.36	0.31	0.22	0.1	0.15	0.08	0.03	0.09	0.08
Fat	0.11	0.22	0.16	0.12	0.06	0.02	0.01	0.01	0.01	0.02
Small intestine	0.81	1.6	0.46	0.44	0.22	0.42	1.1	0.23	0.15	0.14
Large intestine	1.0	0.85	1.3	0.43	0.18	0.39	0.34	0.93	0.18	0.1

TABLE 2.—Levels of vincristine and metabolites in tissues of monkeys given 1 mg/kg iv

Tissue	Vincristine (μg/g) Hrs 1	2	4	8	24	Vincristine metabolites (μg equivalents/g) Hrs 1	2	4	8	24
Brain	0.02	0.01	0.01	0.01	0.01	0.17	0.15	0.18	0.14	0.18
Liver	2.7	1.4	1.2	1.0	0.83	3.0	1.6	1.5	1.3	0.98
Kidney	4.4	3.8	3.5	2.7	0.67	2.3	1.7	0.55	0.95	0.56
Spleen	2.8	2.6	2.1	2.1	1.5	2.2	0.94	2.1	1.8	1.0
Pancreas	3.8	3.7	3.6	3.0	2.8	0.39	0.47	0.41	0.55	0.51
Bone marrow	1.5	0.68	0.7	0.61	0.24	0.42	0.27	0.2	0.22	0.12
Lung	2.6	1.7	1.7	2.2	0.84	0.97	0.44	0.56	0.49	0.43
Heart	1.7	1.1	1.4	1.1	0.5	1.6	0.61	0.68	0.66	0.27
Muscle (gastrocnemius)	0.26	0.47	0.36	0.34	0.12	0.14	0.31	0.27	0.25	0.21
Fat	0.66	0.23	0.29	0.21	0.52	0.21	0.29	0.12	0.09	0.43
Small intestine	1.6	1.5	0.92	0.96	0.55	1.2	1.2	0.55	0.6	0.47
Large intestine	1.4	0.89	1.1	0.89	0.97	1.1	0.41	0.57	0.61	0.69

The accumulation was greater than that expected for the larger dose.

For five dogs and five monkeys given a dose of 1 mg/kg of [^{3}H]vincristine simultaneously with 2 mg/kg of prednisone, the average half-lives were 7 and 6 minutes for the first phase of disappearance and 240 and 235 minutes for the second phase respectively (not shown). These values are not appreciably different from those for animals not given prednisone. The serum levels in dogs were approximately twice as great as those for animals given 0.5 mg/kg. Similarly, the amounts of vincristine in the tissues of dogs were one and one half to two times higher in dogs given the larger dose. The distribution in monkeys was essentially the same as when no prednisone was administered. Again, considerably more vincristine metabolites were found in tissues of monkeys than in tissues of dogs.

Only 10%–25% of the radioactivity in a dose of [^{3}H]vincristine could be recovered in the urine of dogs and monkeys in a 24-hour period; about 60% was unchanged vincristine. Most of the radioactivity excreted was collected in the first 8 hours after drug administration. Results for three dogs indicated that these values were not changed when prednisone was administered simultaneously with vincristine. For two monkeys given the combined dose, however, the excretion of total radioactivity in 24 hours was only 4% and 7%. Again, 60% was present as vincristine. Since a large number of monkeys were not tested, the difference in excretion may not be significant.

About 10% of the total radioactivity was present in the bile of dogs killed at times up to 24 hours; 60% of this was unchanged vincristine. In monkeys killed within 4 hours after injection, the bile con-

tained about 30% of the total radioactivity; about 70% of that present was metabolites of vincristine.

DISCUSSION

One problem in working with tritiated compounds is that the labeled hydrogen atoms may exchange with those of other molecules. Under the conditions used for experiments reported here, there is very little exchange of tritium from vincristine, as indicated by the stability studies and by the small amount of tritiated water in the urine of mice.

The maximum level of [^{3}H]vincristine (0.77 μg/ml) in mouse serum assayed by our procedure is about twofold higher than that (0.36 μg/ml) detected by a cell culture assay after a similar dose (8). The maximum level in rat serum (0.33 μg/ml) is about one half that (0.75 μg/ml) detected by the cell culture assay. The basis for these differences is not apparent, but for the mice, it could be related to the fact that a different strain (Swiss) was used. Other pharmacologic studies in rats show that vincristine is rapidly cleared from the blood and is excreted into the biliary system, intestinal tract, and urine (3,9,10). According to one report, metabolites account for 20% of the amount excreted in the urine of rats in 24 hours after a 0.5-mg/kg dose (9); our results indicate a greater extent of metabolism.

After an iv dose of 1 mg/kg of [^{3}H]vincristine to rats, radioactivity equivalent to 0.1–0.2 μg/g of vincristine accumulates in the kidneys, spleen, and liver at 1 hour (10). Our results with a 2-mg/kg ip dose show that much larger amounts of intact vincristine are present in these tissues. Also, our results for the tissue distribution of vincristine in dogs given a dose of 0.5 mg/kg demonstrate that the amounts of vincristine present in tissues are much greater than values reported (10) for vincristine equivalents.

The disappearance of vincristine from the blood of dogs and monkeys given iv doses is very rapid; the half-lives for the first phase are 6 and 10 minutes respectively. These values are similar to those derived by a biologic assay of blood from dogs and monkeys treated similarly (8). As determined by a newly developed radioimmunoassay for vincristine, the half-lives of the two phases of disappearance in human serum are 5 and 191 minutes respectively (10), values similar to those for dogs and monkeys. The short half-life for the first phase and the high apparent volume of distribution are probably related to the rapid accumulation of the drug by tissues and perhaps to excretion into the bile. The second, longer half-life is probably influenced by urinary excretion and metabolism of vincristine.

For all four species studied in this investigation there is a rapid accumulation of vincristine and its metabolites in most tissues, a phenomenon probably related to intracellular binding of the agent to tubulin and perhaps to other proteins. A notable feature of the tissue distribution studies is the lack of accumulation of appreciable amounts of vincristine in the brains of the animals. To a small extent, mouse and monkey brains accumulate metabolites of vincristine. It remains to be determined if vincristine or one of its metabolites is associated with its neurotoxicity (3). Chemical structures of the metabolites are not known.

A previous paper (9) states that 20% of an unspecified amount of vincristine is converted to metabolic products in 30 minutes by a rat-liver homogenate. Our results with mouse tissue homogenates also indicate that liver is a site of metabolism of vincristine.

The lack of a substantial effect of prednisone on the serum levels, tissue distribution, and excretion of vincristine implies that the synergistic effect of these two agents is not due to altered pharmacokinetics. Results presented in the accompanying manuscript (12) show that vincristine has little effect on the serum levels of prednisone in experimental animals.

The accumulation of vincristine in the pancreas, liver, and kidneys of dogs and monkeys could imply that cancers of these tissues would respond to the drug. Vincristine has not been adequately evaluated against such types of cancer (13); such tests appear to be warranted.

REFERENCES

1. JOHNSON IS, ARMSTRONG JG, GORMAN M, ET AL. The vinca alkaloids: a new class of oncolytic agents. Cancer Res 23:1390-1427, 1963.
2. GOLDIN A, and CARTER SK. Screening and evaluation of antitumor agents. *In* Cancer Medicine (Holland JF, and Frei E III, eds). Philadelphia, Lea and Febiger, 1973, pp 605–628.
3. JOHNSON IS. Plant alkaloids. *In* Cancer Medicine (Holland JF, and Frei E III, eds). Philadelphia, Lea and Febiger, 1973, pp 840–850.
4. HIMES RH, KERSEY RN, HELLER-BETTINGER I, ET AL. Action of the vinca alkaloids vincristine, vinblastine, and desacetyl vinblastine amide on microtubules in vitro. Cancer Res 36:3798–3802, 1976.
5. EL DAREER SM, and MELLETT LB. The estimation of ^{3}H-vincristine (^{3}H-VCR) and its metabolites in biological materials. Pharmacologist 13:294, 1971.
6. MELLETT LB, and EL DAREER SM. Metabolic disposition of ^{3}H-vincristine sulfate (^{3}H-VCR) in various species. Pharmacologist 13:294, 1971.
7. GOLDBAUM LR, and KAZYAK L. Identification of alkaloids and other drugs by paper partition chromatography. Anal Chem 28:1289–1290, 1956.
8. DIXON GJ, DULMADGE EA, MULLIGAN LT, ET AL. Cell culture bioassay for vincristine sulfate in sera from mice, rats, dogs, and monkeys. Cancer Res 29:1810–1813, 1969.

9. CASTLE MC, and MEAD JAR. Investigations of the metabolic fate of tritiated vincristine in the rat by high-pressure liquid chromatography. Fed Proc 35:667, 1976.

10. CASTLE MC, MARGILETH DA, and OLIVERIO VT. Distribution and excretion of [^{3}H]vincristine in the rat and the dog. Cancer Res 36:3684–3689, 1976.

11. OWELLEN RJ, and HAINS FO. Clinical pharmacokinetics of vincristine and vindesine as determined by radioimmunoassay. Proc Am Assoc Cancer Res and ASCO 17:102, 1976.

12. EL DAREER SM, STRUCK RF, WHITE VM, ET AL. Distribution and metabolism of prednisone in mice, dogs, and monkeys. Cancer Treat Rep 61:1279–1289, 1977.

13. WASSERMAN TH, COMIS RL, GOLDSMITH M, ET AL. Tabular analysis of the clinical chemotherapy of solid tumors. Cancer Chemother Rep Part 3, vol 6(2):399–419, 1975.

Distribution and Metabolism of Prednisone in Mice, Dogs, and Monkeys[1,2,3]

Salah M. El Dareer,[4,*] Robert F. Struck,[4] Vivian M. White,[4] L. Bruce Mellett,[4,5] and Donald L. Hill[4,6]

SUMMARY

A chromatographic procedure that allows measurement of [^{3}H]prednisone and [^{3}H]prednisolone in biologic fluids and tissues was developed and used to determine some of the biochemical properties of prednisone and its distribution and metabolism in mice, dogs, and monkeys. When prednisone was added to mouse serum in vitro at a concentration of 50 μg/ml, approximately half of the drug became bound to serum proteins. Homogenates of mouse liver metabolized prednisone to prednisolone. Following ip or oral doses of prednisone to mice, serum levels of prednisone, prednisolone, and other metabolites were maximum at 15 minutes. 20-Dihydroprednisolone was identified as a metabolite in several biologic samples. For dogs and monkeys given an iv dose of prednisone, disappearance from the serum was biphasic with apparent half-lives of 15 and 82 minutes and 33 and 78 minutes respectively. The serum levels of prednisone and prednisolone in mice, dogs, and monkeys were not increased beyond expected levels by simultaneous administration of iv doses of vincristine. The prednisone content of the kidneys, liver, intestine, and bile of a monkey killed 30 minutes after an iv dose was higher than that in serum. Oral doses of prednisone were absorbed erratically by dogs and monkeys. Only small amounts of prednisone and prednisolone were excreted in the urine of dogs and monkeys given an iv or oral dose.

[Cancer Treat Rep 61:1279–1289, 1977]

Prednisone is a synthetic steroid with enhanced glucocorticoid activity (1) and with potent antileukemic activity, especially when combined with vincristine (2,3). A procedure involving radioimmunoassay of prednisolone[7] has been used to measure serum concentrations of this compound in man and dog after oral doses of prednisolone and prednisone (4,5). Prednisolone, the product of an enzymatic reduction of prednisone, is the biologically active form of this agent (6–8). The metabolism of prednisolone has been investigated thoroughly (9–15), but that for prednisone has received less attention (16,17).

This paper describes a procedure for measurement of [^{3}H]prednisone and [^{3}H]prednisolone in biologic fluids and tissues and presents results of studies on the distribution and metabolism of [^{3}H]prednisone in mice, dogs, and monkeys. Preliminary reports of this work have been presented (18,19).

MATERIALS AND METHODS

[6,7(n)-^{3}H]prednisone was purchased from Amersham/Searle Corp, Arlington Heights, Ill, and unlabeled prednisone and prednisolone were obtained from Schwarz-Mann BioResearch, Inc, Orangeburg, NY. Unlabeled vincristine was obtained from Dr.

[1]Received Sept 27, 1976; revised Apr 14, 1977; accepted Apr 20, 1977.

[2]Supported by contract N01-CM-43780 from the Division of Cancer Treatment (DCT), National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3]Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-.

[4]Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Ala.

[5]*Present address:* USV Pharmaceutical Corp, Tuckahoe, NY.

[6]We are grateful to Marion Kirk for performing the mass spectral analyses and to Thomas Herren for assay of radioactive samples.

[7]Prednisolone: NSC-9900; CAS reg. No. 50-24-8; pregna-1,4-diene-3,20-dione, 11β,17,21-trihydroxy-.

**Reprint requests to:* Salah M. El Dareer, DVM, Kettering-Meyer Laboratory, Southern Research Institute, 2000 9th Ave S, Birmingham, Ala 35205.

Harry Wood, Developmental Therapeutics Program, DCT, NCI. Bio-Solv BBS-3 solubilizer was purchased from Beckman Instruments, Palo Alto, Calif, and Liquifluor and Aquasol scintillation solutions from the New England Nuclear Corp, Boston, Mass. All radioactive samples were counted in a liquid scintillation spectrometer equipped with an external standard. Type SA and SG thin-layer sheets were obtained from Gelman Instrument Co, Ann Arbor, Mich, and Analtech silica gel HF plates from Analtech, Inc, Newark, Del. Cellulose dialysis tubing was purchased from Sargent-Welch Scientific Co, Birmingham, Ala.

Unless otherwise indicated, [^{3}H]prednisone (5 millicuries) in ethyl alcohol:benzene (1:1, g/g) was mixed with 500 mg of unlabeled prednisone to give a preparation that, after drying, had a specific activity of 9.19 microcuries/mg. From this stock preparation solutions for injection were prepared at a concentration of 1.0 mg/ml in 50% propylene glycol-water.

The following thin-layer systems were used to determine the radiochemical purity of [^{3}H]prednisone: (*a*) Gelman SA sheets and a solvent of benzene:ethyl alcohol (9:1, vol/vol) (system A); (*b*) Gelman SG sheets and a solvent of chloroform:acetone (4:1, vol/vol) (system B); (*c*) Gelman SA sheets and the first solvent, which was allowed to dry, followed by development with the second solvent (system C); and (*d*) Analtech silica gel plates and a solvent of chloroform:methyl alcohol:water (92:8:0.3, vol/vol/vol) (system D). Unlabeled prednisone and prednisolone, visualized by ultraviolet light at 250 nm, were used as reference compounds. The solvent front was allowed to migrate to 15 cm. After drying, the sheets were cut into 0.6-cm strips, starting 0.6 cm behind the origin and ending about 1 cm beyond the solvent front. The strips were placed in counting vials containing 1 ml of ethyl alcohol for 30 minutes before the addition of 10 ml of Aquasol. Radioactivity migrating with unlabeled prednisone represented the pure compound. As judged with these systems, the purity of [^{3}H]prednisone was 98%–100%. Prednisone had R_f values of 0.40–0.45, 0.70–0.75, 0.80–0.85, and 0.55–0.60 in systems A, B, C, and D respectively.

To measure protein binding of prednisone, cellulose dialysis tubing was cut to a length of 15 cm, a double knot was tied in one end, and the top was fitted over the top of a sintered-glass filtration funnel with a shortened stem. The funnel was equipped with a rubber stopper and inserted into a cup prepared from a 35-ml centrifuge tube. After the serum-drug solution was added to the bag, the apparatus was centrifuged at 2000 × *g* for 16 hours at 4°C. Radioactivity in the solution passing through the membrane was measured and the percent of binding was calculated. Adsorption to the tubing and to the glass was determined by using aqueous solutions of prednisone.

Animals used in this study were male BDF_1 mice weighing 25–30 g, female beagle dogs weighing 10–13 kg, and female rhesus monkeys weighing 4.5–6.0 kg. All animals were starved overnight prior to use. Urine was collected by catheters. Blood was collected from the axillary region of mice rendered unconscious with CO_2 shortly before collection and from the anterior radial or saphena veins of monkeys and dogs. Various organs were collected, blotted on filter paper, pooled according to type, placed on solid CO_2, and homogenized, after thawing, in four volumes of ethyl alcohol with a Brinkmann Polytron equipped with a P-10 generating head (Brinkmann Instruments, Westbury, NY). At the time of collection the contents of intestines were removed.

For determination of total radioactivity in biologic samples, 15 ml of Liquifluor containing Bio-Solv BBS-3 (14:1, vol/vol) was added to a 0.1-ml portion prior to scintillation counting. Although the method was not used routinely, 99%–101% of the radioactivity present in biologic samples could be recovered after precipitation of proteins by adding three volumes of 95% ethyl alcohol and centrifuging.

For separation of [^{3}H]prednisone and its products, system C was used for portions (5–200 μl) of supernatant preparations, and standards of prednisone and prednisolone were spotted as reference compounds. Urine samples were spotted directly onto thin-layer sheets. The sheets were cut into strips and counted as described above.

Mass spectral analyses were performed with a Hitachi mass spectrometer, model RMU-6-D.

RESULTS

Stability and Protein Binding of [^{3}H]Prednisone

[^{3}H]prednisone was prepared as a solution of 100 μg/ml in sterile 0.9% sodium chloride containing 5% propylene glycol. Portions of this solution were incubated at 37°C for 1 week without any degradation products appearing on thin-layer sheets. However, when a similar solution of 50 μg/ml of [^{3}H]prednisone was heated in a sealed vial for 6 hours at 100°C in a test for stability under severe conditions, 47% of the radioactivity appeared as degradation products.

[^{3}H]prednisone was added to mouse liver and kidney homogenates prepared in four volumes of 0.9% NaCl, and to mouse serum at a concentration of 100 μg/ml; the preparations were maintained at

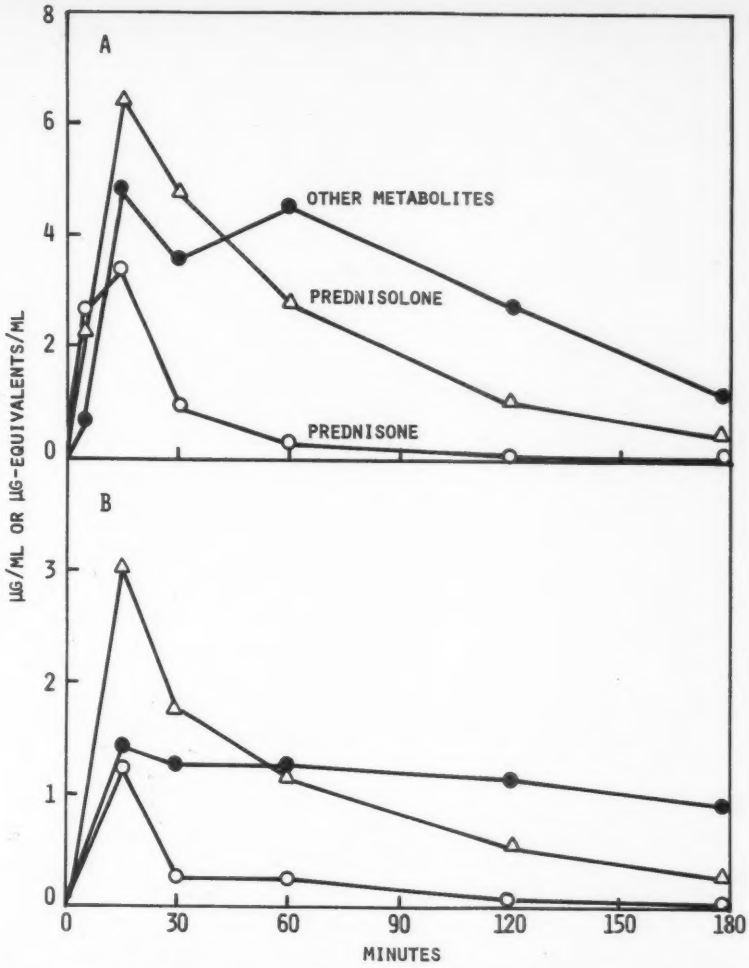


FIGURE 1.—Levels of prednisone, prednisolone, and other metabolites in the serum of mice given ip (A) or oral (B) doses of 10 mg/kg of [^{3}H]prednisone.

37°C. At intervals up to 2 hours, portions were analyzed by thin-layer chromatography (system C). During this time, no detectable prednisolone appeared in the serum or kidney homogenate; but, in the liver homogenate at 2 hours, 64% of the radioactivity was present as prednisolone, 31% as prednisone, and 5% as other, unidentified products. The identity of prednisone and prednisolone from these preparations was confirmed by mass spectral analysis.

Binding of [^{3}H]prednisone to serum proteins was determined by filtration of solutions of 50 µg/ml in serum through cellulose dialysis membranes at 4°C. For mouse and dog serum, 43% and 50% respectively passed through the membrane; the remainder was apparently bound to serum proteins. When no serum was present, 100% of the radioactivity passed through the membrane.

[^{3}H]prednisone in Mice

An ip dose of 10 mg/kg of [^{3}H]prednisone was administered to one group of mice. Serum and tissues from five mice were pooled for each determination of prednisone and its metabolites. Serum levels of unchanged prednisone were maximum (3.4 µg/ml) at 15 minutes (fig 1A); very little prednisone was present after 1 hour. Prednisolone appeared in

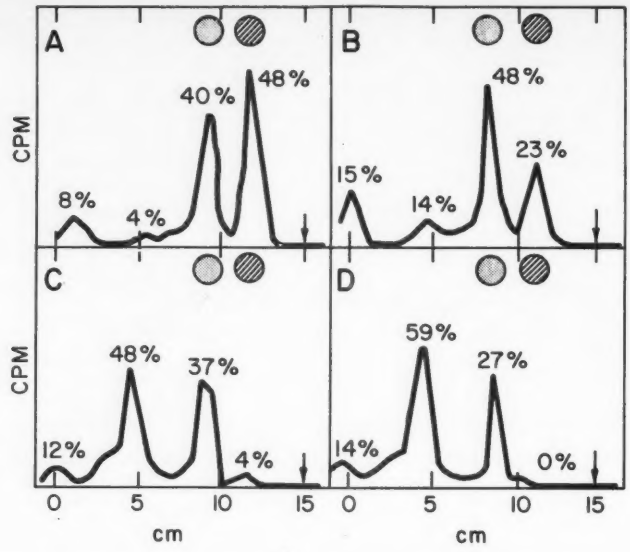


FIGURE 2.—Thin-layer chromatography of serum of mice given an ip dose of 10 mg/kg of [^{3}H]prednisone. A = 5 mins after injection; B = 15 mins after injection; C = 60 mins after injection; D = 120 mins after injection. The migration of standard prednisone and prednisolone is indicated by the cross-hatched and stippled circles respectively. The figures are plotted on scales indicating the percent of total counts present.

the serum at its highest concentration, 6.4 μg/ml, 15 minutes after prednisone administration; the amount fell rapidly after that time. The concentration of other metabolites in mouse serum did not vary greatly between 15 and 60 minutes. When vincristine (3 mg/kg) was administered ip to mice simultaneously with 10 mg/kg of [^{3}H]prednisone, assays of serum prednisone and prednisolone revealed curves (not shown) similar to those for figure 1A.

After an oral dose of 10 mg/kg of [^{3}H]prednisone, levels of prednisone and prednisolone in mouse serum, although lower than after an ip dose, were also maximum at 15 minutes (fig 1B). (In this case, 15 minutes was the first time of assay.) The concentration of other metabolites did not vary greatly between 15 and 180 minutes.

Figure 2 illustrates a typical separation of prednisone and its metabolites in mouse serum using solvent C. The identity of prednisone and prednisolone, as eluted from these chromatograms, was confirmed by mass spectral analysis in comparison with authentic samples. Elution of the peaks containing prednisone and prednisolone and rechromatography of the samples in solvent systems containing benzene:ethyl alcohol (9:1, vol/vol) and chloroform:ethyl alcohol (9:1, vol/vol) showed that the peaks contained < 5%–9% of other compounds. Metabolites other than prednisolone appeared on the chromatograms at the origin and with R_f values of 0.3 and 0.4. The major component at R_f 0.4 was identified as 20-dihydroprednisolone by mass spectral analysis. In comparison with an authentic sample of this compound, the molecular ion (m/e 362) was observed in addition to a major fragment ion (m/e 301), corresponding to a loss of $[-CH(OH)CH_2OH]^+$ and confirming reduction at C-20.

In the livers of mice given an ip dose of [^{3}H]prednisone, the agent was rapidly converted to prednisolone and other metabolites (fig 3A). After 5 minutes, only 8% of the radioactivity present in this organ was unchanged prednisone. At this time, prednisolone, at a concentration of 21 μg/g, accounted for 50% of the total radioactivity. The prednisolone levels remained high for > 30 minutes; but, after 15 minutes, most of the radioactivity in the liver was present as unidentified metabolites. Following an oral dose of [^{3}H]prednisone, the liver contents of prednisone and prednisolone were about half those following the ip dose (not shown). The amount of other metabolites was essentially the same as for the ip dose during the 3-hour observation period.

In kidney tissue, unchanged prednisone reached its highest concentration (3.2 μg/g) at 15 minutes

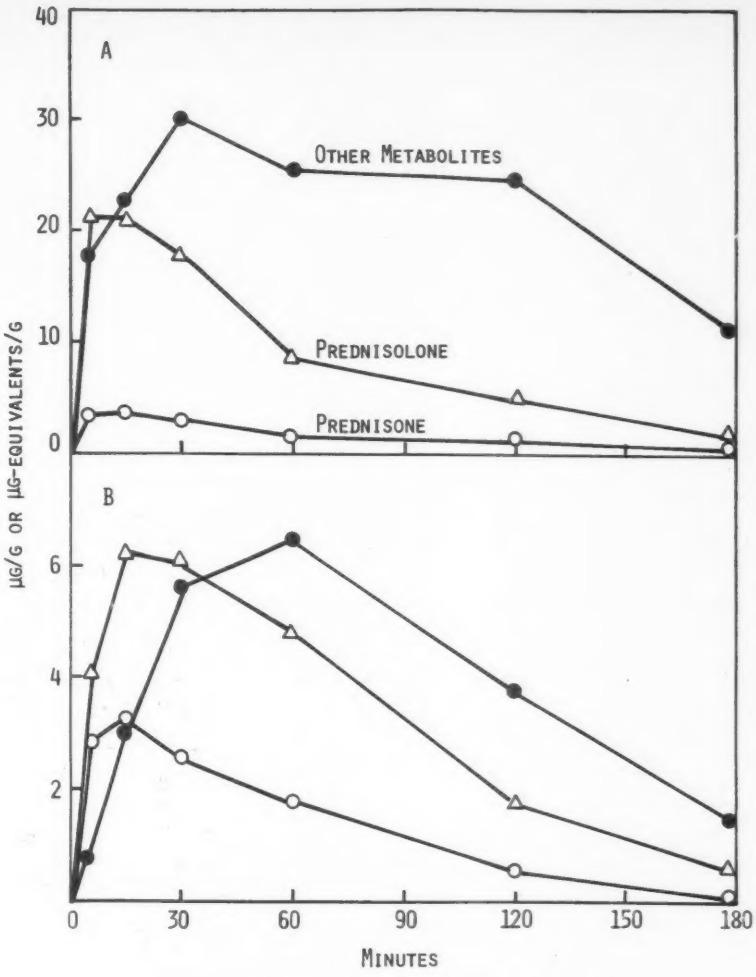


FIGURE 3.—Levels of prednisone, prednisolone, and other metabolites in the liver (A) and kidneys (B) of mice injected ip with 10 mg/kg of [^{3}H]prednisone.

following an ip dose; no detectable prednisone was present at 3 hours (fig 3B). Prednisolone concentrations followed a similar pattern, with levels about twice those for prednisone. As observed for the kidneys, liver levels of prednisone and prednisolone following an oral dose were about half the values following an ip dose (not shown). The levels of other metabolites were similar to those for ip doses.

In 24 hours after an ip dose of 10 mg/kg of [^{3}H]prednisone, mice excreted 30% of the total radioactivity in the urine; only 4% was excreted between 24 and 48 hours. For both periods, < 12% of the radioactivity in the urine represented prednisone plus prednisolone. After an oral dose of 10 mg/kg, mice also excreted in the urine 30% of the total radioactivity in 24 hours. However, prednisone and prednisolone accounted for 25% of the amount excreted.

Thin-layer chromatography of a chloroform extract of mouse urine (0–25 hours) obtained after treatment of the mice with an ip dose of 10 mg/kg of prednisone demonstrated the presence of approximately equal amounts of prednisolone and 20-dihydroprednisolone and a lesser amount of prednisone. Identification of metabolites was based on comparison in two thin-layer systems (C and D).

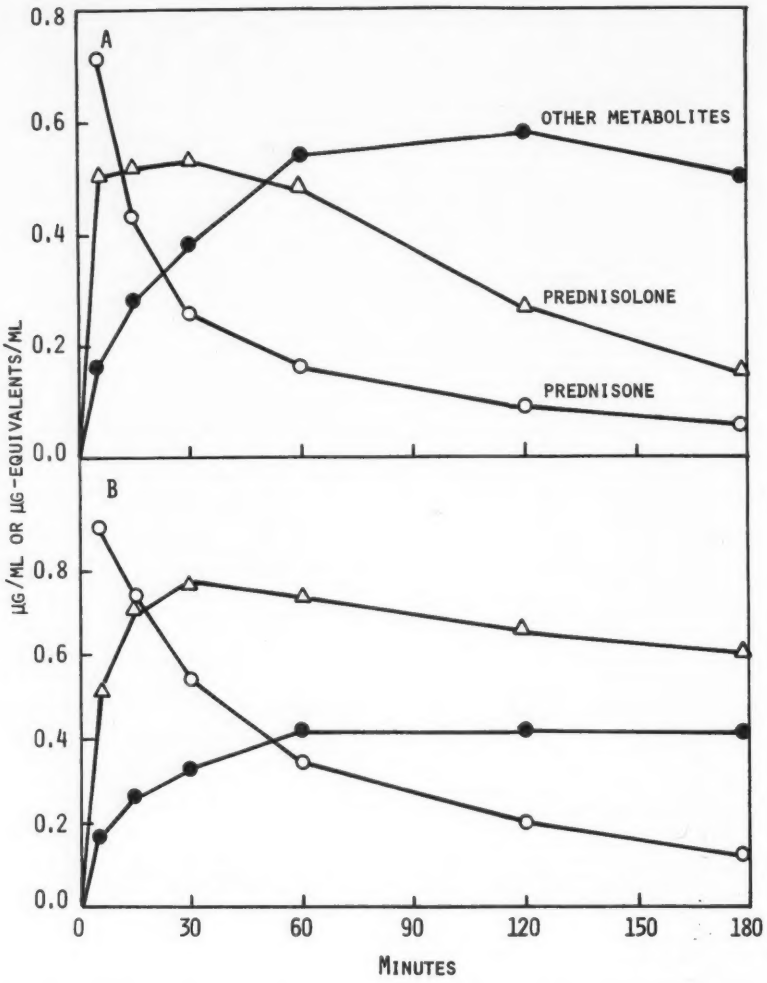


FIGURE 4.—Levels of prednisone, prednisolone, and other metabolites in the serum of dogs (A) and monkeys (B) given an iv dose of 1 mg/kg. Values for 2 dogs and 2 monkeys were averaged to give these results.

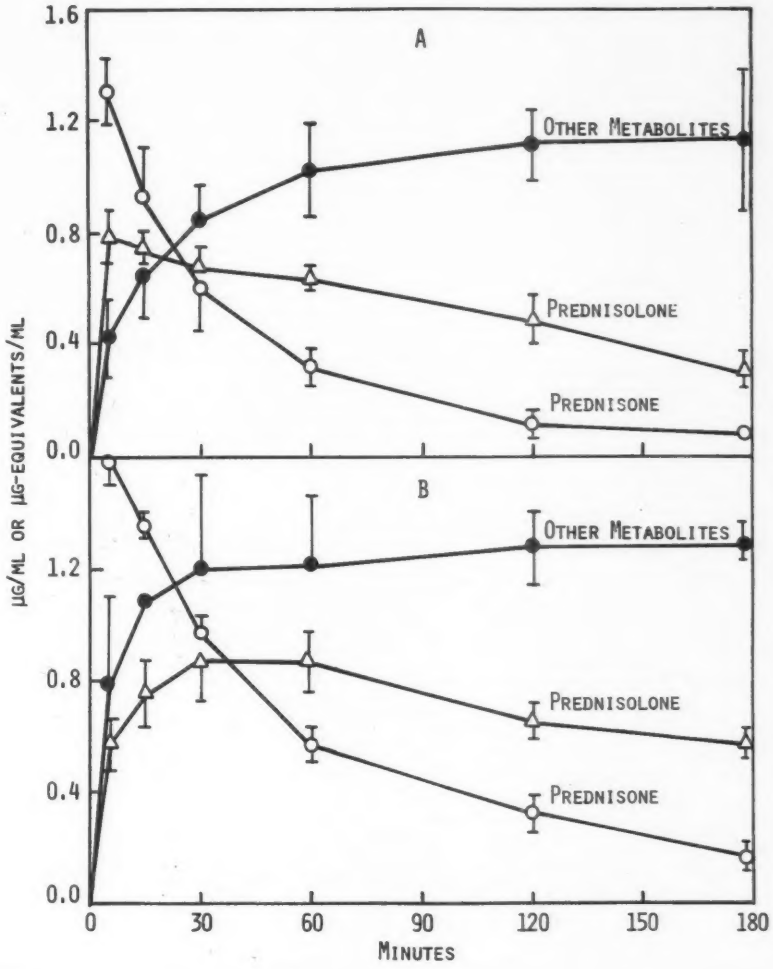


FIGURE 5.—Levels of prednisone, prednisolone, and other metabolites in the serum of dogs (A) and monkeys (B) given an iv dose of 2 mg/kg of [^{3}H]prednisone and 1 mg/kg of vincristine. The value for other metabolites in the serum of monkeys at 15 mins is 1.08 ± 0.46 µg/ml; the standard deviation could not conveniently be added to the figure.

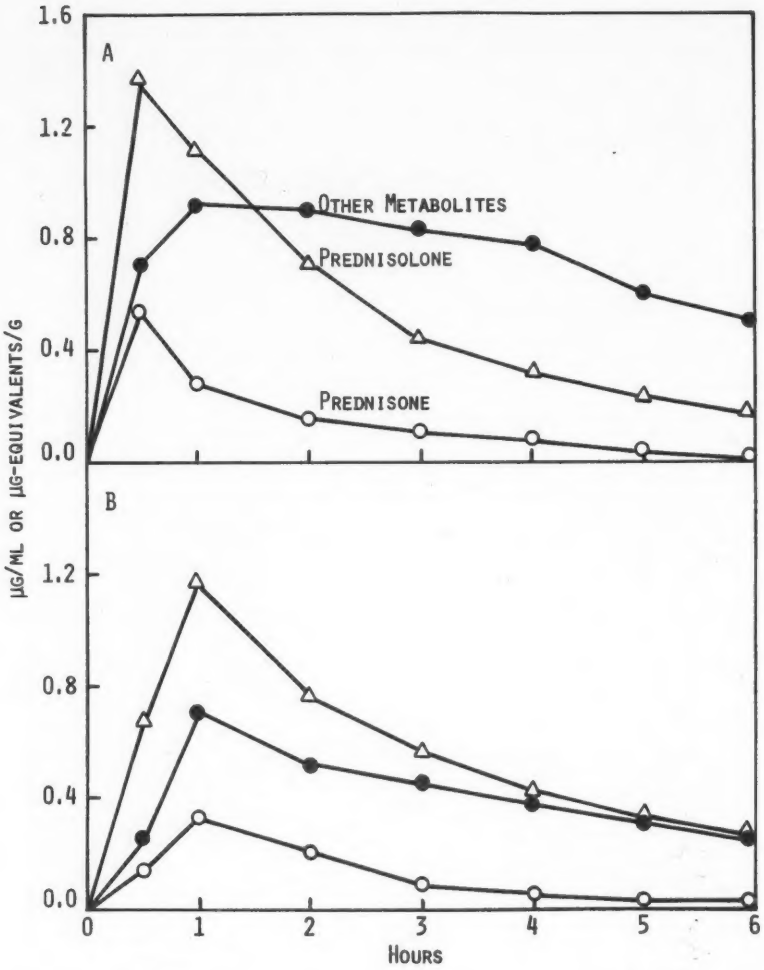


FIGURE 6.—Levels of prednisone, prednisolone, and other metabolites in the serum of a dog (A) and a monkey (B) given an oral dose of 2 mg/kg of [^{3}H]prednisone.

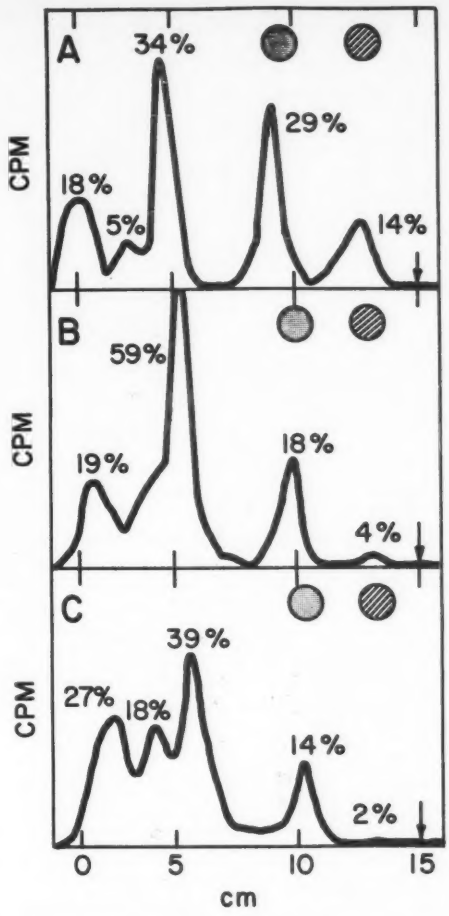


FIGURE 7.—Thin-layer chromatography of the urine of dogs given an iv dose of 2 mg/kg of [^{3}H]prednisone. A = urine collected in the period 0–4 hrs after injection; B = 4–6 hrs; C = 6–24 hrs. The symbols and scales are the same as for fig 2.

[^{3}H]prednisone in the Dog and Monkey

The iv injection of 1 mg/kg of [^{3}H]prednisone into two dogs resulted in an average serum level for this agent of 0.71 μg/ml at 5 minutes (fig 4A). The apparent half-life for the first phase of elimination was 15 minutes and that for the second phase was 82 minutes (not shown). The prednisolone concentration remained almost constant for 1 hour before dropping. The concentration of other metabolites in the serum increased for 1 hour, then remained at a high level for the next 2 hours.

For two monkeys injected iv with 1 mg/kg of [^{3}H]prednisone, the average serum content of the drug was 0.87 μg/ml at 5 minutes (fig 4B). Prednisolone disappeared from the serum with an apparent half-life of 33 minutes for the first phase and 78 minutes for the second phase (not shown). The prednisolone level was maximum between 15 and 60 minutes and dropped slowly after that time. The level of other metabolites rose to a maximum at 60 minutes and remained near that amount throughout the remainder of the experiment.

Three dogs were injected with a larger dose of [^{3}H]prednisone (2 mg/kg) and simultaneously with 1 mg/kg of vincristine. Both drugs were administered iv. At 5 minutes the serum level of prednisone was 1.31 ± 0.10 μg/ml (fig 5A). The average apparent half-life for the first phase was 20 minutes and that for the second phase was 100 minutes (not shown). Again, the prednisolone concentration remained almost constant for 1 hour before dropping. The concentration of other metabolites rose in the serum for 60 minutes and then remained relatively constant.

Three monkeys were also injected with the larger dose of prednisone (2 mg/kg) and with vincristine (1 mg/kg) (fig 5B). At 5 minutes, the serum concentration of prednisone was 1.68 ± 0.10 μg/ml. As determined by plotting these data on a logarithmic scale (not shown), the apparent half-lives were 30 minutes for the first phase and 100 minutes for the second phase. As when prednisone was given alone, the prednisolone concentration was maximum between 15 and 60 minutes and dropped slowly after that time. The level of other metabolites rose to a high level at 30 minutes and remained high for the duration of the experiment.

For dogs and monkeys given an iv dose of prednisone, the apparent volumes of distribution were 30 and 9 liters respectively—values unreasonably high for uniform distribution of the drug.

The results obtained after an oral dose of 2 mg/kg to each of two dogs differed in the time for maximum serum concentrations of prednisone, prednisolone, and other metabolites. The results for one dog are in figure 6A. For the other dog, the maximum serum concentrations of prednisone (0.19 μg/ml) and prednisolone (0.87 μg/ml) were reached at 1 hour after dosing, and the concentration of other metabolites was highest (1.38 μg/ml) at 2 hours (not shown). For both dogs, very little prednisone was present in the serum after 3 hours and very little prednisolone at 5 hours. The concentration of other metabolites in the serum remained high between 1 and 6 hours.

For two monkeys given an oral dose of 2 mg/kg of [^{3}H]prednisone, the results were also different. Data

obtained for one monkey are shown in figure 6B. For the other monkey, serum levels of all three measured components were highest at 2 hours (not shown). In both cases, there was a gradual drop in the concentrations of prednisolone and other metabolites between 2 and 6 hours; very little prednisone was present after 3 hours.

Total urinary excretion of radioactivity by the two dogs injected iv with 1 mg/kg of [^{3}H]prednisone was 63% and 69% of the total dose in 24 hours. Of the total dose, 2% was excreted as prednisone and 6% as prednisolone. The remaining material in the urine was other metabolites. For the two dogs given 2 mg/kg of [^{3}H]prednisone orally, 61% and 72% of the radioactivity appeared in the urine within 24 hours. Of the total dose, an average of 2% was excreted as prednisone and an average of 6% as prednisolone.

Another dog was injected iv with 2 mg/kg of [^{3}H]prednisone of higher specific activity (41.7 microcuries/mg), and urine samples were collected for the periods 0–4, 4–6, and 6–24 hours (table 1). Prednisone accounted for 4% of the total dose and prednisolone for 11%. Separation of prednisone and its metabolites in these samples is illustrated in figure 7. More polar metabolites formed with increasing time after the dose. From methyl alcohol extracts of samples, 20-dihydroprednisolone was isolated by thin-layer chromatography (system D) and identified by mass spectral analysis by comparison with an authentic sample. For each period, 20-dihydroprednisolone was the major metabolite excreted, accounting for more than one half of the radioactivity listed as "other metabolites." 20-Dihydroprednisolone was also detected in the blood of this dog.

Only small amounts of prednisone and prednisolone were excreted in the urine of monkeys. Of the total dose given, prednisone and prednisolone excretion was an average of 7% and 11% respectively after the iv dose of prednisone and 2% and 16% respectively after oral doses. Total recovery of radioactivity in the urine at 24 hours was 68% and 84% for the two monkeys.

One monkey, injected iv with 1 mg/kg of [^{3}H]-prednisone, was killed 30 minutes later and its tissues were examined for their content of prednisone, prednisolone, and other metabolites. The results are in table 2. The kidneys contained the largest amounts of prednisone and other metabolites, and the liver contained the largest concentration of prednisolone. The prednisone content in the kidneys, liver, intestine, and bile was appreciably higher than that in the serum.

TABLE 1.—Recovery of prednisone and its metabolites in the urine of a dog given an iv dose of 2 mg/kg of [^{3}H]prednisone

Time of collection (hrs)	% of total dose		
	Prednisone	Prednisolone	Other metabolites
0–4	3.35	6.94	13.63
4–6	0.33	1.47	6.37
6–24	0.37	2.61	15.65

TABLE 2.—Distribution of [^{3}H]prednisone and its metabolites in tissues and body fluids of a monkey 30 mins after iv administration of 1 mg/kg

Body fluid	μg/g or μg equivalents/g		
	Prednisone	Prednisolone	Other metabolites
Brain	<0.10	<0.10	<0.10
Liver	0.59	2.84	2.48
Kidney	2.28	1.06	4.26
Spleen	0.41	0.60	0.21
Pancreas	0.38	0.88	0.19
Bone marrow	0.14	0.45	0.29
Lung	0.25	0.82	0.32
Muscle	0.26	0.29	0.13
Heart	0.19	0.80	0.30
Small intestine	0.78	0.80	0.78
Large intestine	0.68	0.40	0.36
Colon	0.54	0.21	0.10
Fat	0.26	0.20	0.10
Serum*	0.36	0.63	0.40
Bile*	1.41	0.50	8.18

*μg/ml or μg equivalents/ml.

DISCUSSION

The method used in this study for assay of prednisone and prednisolone in biologic materials has advantages over the radioimmunoassay for prednisolone (4) in that both compounds are measured directly and that the time required for assay is shorter. The chromatographic procedure is simpler than that used for plasma estriol assays (20).

Our results show that prednisone is readily converted to prednisolone and other metabolites in mice, dogs, and monkeys. The same major metabolites are formed by all three species. The metabolism apparently takes place primarily in the liver. The large amounts of 20-dihydroprednisolone in the urine of dogs indicate a quantitative difference between dogs and humans, who excrete only small amounts of this derivative (21). The other products are not identified, but may be any of several other prednisone or prednisolone metabolites previously described (9,10,22).

The relative amounts of prednisone, prednisolone, and other metabolites in the serum of mice, dogs, and monkeys at various times after administration of prednisone are generally similar, regardless of

the route of administration. The concentration of prednisone is either low initially or drops rapidly to low levels. The concentration of prednisolone rises within a few minutes to levels greater than those for prednisone and then begins to decline. The concentration of other metabolites rises and falls more slowly than that of prednisolone.

Serum levels of prednisone, prednisolone, and other metabolites in mice given ip doses of prednisone are higher than for those given oral doses. In both cases, peak serum levels occur at 15 minutes. For dogs and monkeys given oral doses of 2 mg/kg of prednisone, serum levels of prednisone and prednisolone are about twice those in animals given iv doses of 1 mg/kg. Oral doses given to dogs and monkeys are apparently erratically absorbed, as revealed by the differences between individual animals.

The high apparent volume of distribution of prednisone after iv doses to dogs and monkeys is probably related to the rapid metabolism of the drug and to its accumulation in the kidneys, liver, intestine, and other tissues. However, at 15 minutes after an ip dose of prednisone to mice, there is no appreciable excess of intact prednisone in the liver or kidneys, compared to serum.

The synergistic effect of prednisone and vincristine against leukemic cells could have a pharmacologic basis because the administration of vincristine could lead to higher blood levels of prednisone and/or prednisolone. However, our studies show no evidence of such an effect. Further, data in the accompanying manuscript (23) indicate that prednisone, administered simultaneously, does not greatly alter the pharmacokinetics and distribution of vincristine in experimental animals.

REFERENCES

1. TALLEY RW. Corticosteroids. *In* Cancer Medicine (Holland JF, and Frei E III, eds). Philadelphia, Lea and Febiger, 1973, pp 933–945.
2. AUR RJA, SIMONE J, HUSTU HO, ET AL. Central nervous system therapy and combination chemotherapy of childhood lymphocytic leukemia. Blood 37:272–281, 1971.
3. HENDERSON ES. Acute lymphoblastic leukemia. *In* Cancer Medicine (Holland JF, and Frei E III, eds). Philadelphia, Lea and Febiger, 1973, pp 1173–1199.
4. COLBURN WA, and BULLER RH. Radioimmunoassay for prednisolone. Steroids 21:833–846, 1973.
5. DISANTO AR, and DESANTE KA. Bioavailability and pharmacokinetics of prednisone in humans. J Pharm Sci 64:109–112, 1975.
6. JENKINS JS, and SAMPSON PA. Conversion of cortisone to cortisol and prednisone to prednisolone. Br Med J 2:205–207, 1967.
7. LEWIS GP, JUSKO WJ, BURKE CW, ET AL. Prednisone side-effects and serum-protein levels. Lancet 2:778–781, 1971.
8. D'ARCY PF, GRIFFIN JP, JENKINS JS, ET AL. Sustained-release formulation of prednisolone administered orally to man. J Pharm Sci 60:1028–1033, 1975.
9. CASPI E, and PECHET MM. Metabolism of 1-dehydrosteroids in man. I. Isolation of six urinary products after the administration of prednisolone. J Biol Chem 230:843–851, 1958.
10. VERMEULEN A, and CASPI E. Metabolism of prednisolone by homogenates of rat liver. J Biol Chem 233:54–56, 1958.
11. VERMEULEN A. Metabolism of prednisolone-4-C^{14}. J Endocrinol 18:278–291, 1959.
12. GLENN EM. In vitro and in vivo metabolism of prednisolone: studies concerning its biological effectiveness. Endocrinology 64:373–378, 1959.
13. BAILEY E, and WEST HF. Prednisolone metabolism and rheumatoid arthritis. Lancet 2:1231–1232, 1967.
14. TAMM J, VOIGT KD, VOLKWEIN U, ET AL. Water-soluble steroid conjugates. II. Metabolism of prednisone 21-glycinate and of cortisol 21-sulfate in the perfused dog liver (comparison with prednisone and cortisol). Steroids 2:279–295, 1963.
15. YAMASHITA O. Metabolism of synthetic corticoids in rat liver. Nippon Naibumpi Gakkai Zasshi 47:1033–1045, 1972. (Chem Abstr 77:83767u, 1972)
16. SCHRIEFENS H, KORUS W, and DIRSCHERL W. Comparison of the metabolism of cortisone and other antirheumatic steroids in slices of rat livers. Acta Endocrinol 26:331–344, 1957.
17. HAJOS GT, SZPORNY L, and TUBA Z. 11-Oxido-reduction of some natural and synthetic glucocorticoids in rat tissue in vitro. Steroids 15:449–458, 1970.
18. EL DAREER SM, MELLETT LB, and WHITE VM. The metabolic disposition of [^{3}H]prednisone in BDF_1 mice. Pharmacologist 17:266, 1975.
19. EL DAREER SM, WHITE VM, DILLON DL, ET AL. Metabolic disposition of ^{3}H-prednisone in dogs and monkeys. Pharmacologist 18:127, 1976.
20. GOEBELSMANN V, and GORWILL RH. The use of silica gel impregnated glass fiber sheets in plasma estriol assays. Steroids 16:635–647, 1970.
21. BUSH IE, and MAHESH VB. Metabolism of 11-oxygenated steroids. 3. Some 1-dehydro and 9α-fluoro steroids. Biochem J 93:236–255, 1964.
22. NAKAGAWA T. Metabolism of [^{14}C]prednisolone and [^{14}C]prednisone in healthy adult men. Kyoto Furitsu Ika Daigaku Zasshi 81:145–159, 1972. (Chem Abstr 77:29310b, 1972)
23. EL DAREER SM, WHITE VM, CHEN FP, ET AL. Distribution and metabolism of vincristine in mice, rats, dogs, and monkeys. Cancer Treat Rep 61:1269–1277, 1977.

5-Fluorouracil-Induced Increase of Total Serum Thyroxine and Triiodothyronine [1,2]

L. Beex,* A. Ross, A. Smals, and P. Kloppenborg [3,4]

SUMMARY

Total serum thyroxine and triiodothyronine levels in a group of 15 patients with advanced mammary carcinoma significantly increased during weekly treatment with 5-fluorouracil, whereas serum free thyroxine levels and serum thyroid stimulating hormone levels were unchanged. No clinical signs of hyperthyroidism in these treated patients have been observed.

[Cancer Treat Rep 61:1291–1295, 1977]

During long-term treatment of patients with advanced breast cancer with 5-fluorouracil (5-FU) as a single drug it was by chance observed that total serum thyroxine (TT_4) levels rose to values comparable to those obtained in patients with moderate or severe hyperthyroidism. None of these patients, however, showed clinical signs of this disorder. This report accounts for this finding and included are a number of data, admittedly retrospective in part, which strongly suggest that increased capacity of the serum to bind thyroid hormone is the cause of increased total thyroid hormone levels in patients receiving long-term treatment with 5-FU.

PATIENTS AND METHODS

The effect of 5-FU treatment on serum TT_4 was evaluated in 15 postmenopausal or ovariectomized women with advanced breast cancer. All were in fairly good and stable condition without discernible impairment of liver and kidney function during the study period. 5-FU in a dose of 15 mg/kg was given once a week by iv injection. No other therapeutic agents were added or omitted during the observation period, nor did these patients have any hormonal treatment. Venous blood for estimation of thyroid hormone levels was sampled at 4-week intervals before and during treatment, and in three patients also after stopping 5-FU treatment. During treatment, blood was taken immediately before administration of 5-FU, ie, 1 week after the previous injection.

In subgroups of patients, total serum triiodothyronine (TT_3) and thyroid stimulating hormone (TSH) levels were measured before and during 5-FU therapy. Furthermore, in three of these patients determinations of free thyroxine percentage ($fT_4\%$) and serum free thyroxine (AfT_4) levels before and during treatment were made. Free thyroxine levels only during 5-FU treatment were also available in one other patient of this group and three additional women with breast cancer receiving treatment with 5-FU.

TT_4 was measured by a modification of the Murphy-Jachan assay (1) using tetrahydrofuran to extract the hormone quantitatively. ^{125}I-labeled thyroxine, specific activity 50 millicuries/mg, was obtained from the Radiochemical Centre (Amersham, England). In this laboratory the normal range for TT_4 is 5.0–11.5 μg/100 ml. The inter-assay replicate variation was 8.1% (n = 29) and the intra-assay duplicate variation was 4.0% (n = 29).

TT_3 was determined by direct radioimmunoassay, using an antiserum obtained by immunizing rabbits against a bovine serum albumin conjugate of triiodothyronine which was prepared according to Gharib et al (2). Final dilution of antiserum ranged from 1:12.000 to 1:16.000. ^{125}I-labeled triiodothyron-

[1]Received Nov 10, 1976; revised June 6, 1977; accepted June 13, 1977.

[2]5-Fluorouracil: NSC-19893; CAS reg. No. 51–21–8.

[3]Division of Endocrinology, St. Radboud Hospital, University of Nijmegen, The Netherlands.

[4]We gratefully acknowledge Dr. R. Lequin for the thyroid stimulating hormone radioimmunoassays, and Mr. J. Willems for skillful technical assistance.

*_Reprint requests to:_ L. Beex, MD, Department of Medicine, Division of Endocrinology, St. Radboud Hospital, University of Nijmegen, Nijmegen, The Netherlands.

ine, at a specific activity of 400–500 millicuries/mg, was obtained from Abbott Laboratories (Germany). Bound and free fractions were separated by means of a second antibody, coupled to cellulose (DASP, Organon, Oss, The Netherlands). The normal values in this laboratory range from 85 to 180 ng/100 ml. The inter-assay replicate variation was 5.2% (n = 7), and the intra-assay duplicate variation was 7.7% (n = 110).

5-FU added in vitro in amounts ranging from 250 to 2500 μg/ml of serum did not affect either TT_4 or TT_3 levels measured. Moreover, in six patients, TT_4 and TT_3 levels were measured in blood taken immediately before and 5 minutes after injection of 5-FU. The results obtained did not differ significantly (Ross A, unpublished data).

TSH was determined by a heterologous radioimmunoassay system using antibodies to porcine TSH and human TSH as the ^{125}I-labeled hormone (3). In the range of the TSH standard curve (2–100 microunits/ml of plasma), highly purified preparations of LH, FSH, and HCG do not react in this system. Bound and free fractions were separated by a double antibody solid phase method (DASP, *see* above). In a group of normal, healthy persons TSH levels ranged from not detectable, < 2.0 microunits/ml, to approximately 10.0 microunits/ml. The inter-assay replicate variation was 9.5% (n = 23), and the intra-assay duplicate variation was 10.4% (n = 23).

The fT_4% was determined by means of equilibrium dialysis as described by Herrmann and Krüskemper (4) with magnesium chloride precipitation as the final purification step. Normal values ranged from 0.014% to 0.030% (n = 16). AfT_4 was obtained by multiplication of the results of TT_4 and fT_4% measurements. Normal values ranged from 1.21 to 2.16 ng/100 ml (n = 16).

Thyroid hormone and TSH levels were determined in separate assay series, usually within 1 week after sampling.

Changes of TT_4, TT_3, and TSH levels during 5-FU treatment were statistically evaluated using the sign test ($P^{\times}$) and the mean value of Spearman rank correlation coefficients ($P^{\times\times}$). The F test for the ratio of variances was used to compare the duplicate variance of pretreatment TT_4 values with the inter-assay variance of the method ($P^{\times\times\times}$).

RESULTS

Pretreatment TT_4 levels ranged from 6.4 to 10.8 μg/100 ml (table 1). In nine patients, TT_4 was measured twice with an interval ranging from 1 to 4 weeks. The values did not differ significantly ($P^{\times} > 0.2$). The standard deviation (SD) calculated from these nine pairs did not differ significantly from the SD representing the between assay variation of the TT_4 assay ($P^{\times\times\times} > 0.2$).

During weekly treatment with 5-FU, the mean

TABLE 1.—Rise of serum TT_4 levels during 5-FU treatment

Patient No.	Previous treatment*	TT_4 level before treatment with 5-FU (μg/100 ml)	TT_4 level (μg/100 ml) during 5-FU treatment at Week—								
			4	8	12	16	20	24	28	32	36
1	R	10.1	—	13.2	12.3	12.4	11.6	13.9	—	—	—
Second course	R	8.4	9.2	16.1	—	—	—	—	—	—	—
2	R	10.7 8.8	15.5	14.9	—	—	—	—	—	—	—
Second course	M, R	10.0	—	12.5	—	14.7	20.6	—	—	—	—
3	M, R	8.6 8.0	10.5	14.2	—	—	—	—	—	—	—
4	R	10.3	9.5	11.8	10.8	12.8	—	14.6	15.0	13.2	14.4
5	M, R	10.0	11.6	14.8	16.5	16.3	13.6	14.8	14.0	13.3	15.1
6	R	9.7	11.1	11.7	—	11.9	11.4	11.8	10.9	11.9	13.2
7	R, O	7.6 7.7	8.0	9.7	13.2	12.9	15.4	—	—	—	—
8	M, O	10.4 10.1	—	9.2	11.6	—	13.8	12.0	13.2	10.5	12.7
9	R	8.2 10.5	10.8	13.3	13.4	14.4	14.4	13.7	14.8	15.9	14.9
10	M, R	9.6 10.4	10.1	15.8	14.8	17.3	12.5	12.1	—	—	—
11	M, R	10.4	10.9	15.6	15.0	16.5	—	—	—	—	—
12	M, R	6.6 6.5	8.3	9.7	—	—	—	—	—	—	—
13	O	7.9 9.0	12.5	14.6	17.0	—	13.8	14.9	—	—	—
14	R	6.8 6.4	10.0	7.8	7.0	8.4	—	—	—	—	—
15	R	10.8	12.6	15.4	19.0	20.7	17.5	20.6	—	—	—
Mean TT_4		8.5 9.2	10.8	13.0	13.6	14.4	14.5	14.3	13.6	13.0	14.1
± SD		1.5 1.4	1.9	2.6	3.3	3.3	2.8	2.7	1.7	2.0	1.1

*M = mastectomy; R = radiotherapy; O = ovariectomy.

TABLE 2.—Rise of serum TT_3 levels during 5-FU treatment

Patient No.	Previous treatment*	TT_3 level before treatment with 5-FU (ng/100 ml)		TT_3 level (ng/100 ml) during 5-FU treatment at Week—				
				4	8	12	16	20–36 (mean values)
2	M, R		131	—	179	—	167	173
8	M, O	151	153	—	—	272	—	203
9	R		192	—	—	—	230	259
10	M, R		115	133	157	147	191	180
11	M, R		156	140	228	231	193	—
12	M, R	101	100	127	102	—	—	144
13	O	201	200	213	240	162	—	223
14	R	152	140	—	130	—	139	—
15	R		186	—	248	221	222	218
Mean TT_3		151.3	152.1	153.3	183.4	206.6	190.3	200.0
± SD		40.8	35.0	40.2	57.1	51.5	34.0	37.9

*M = mastectomy; R = radiotherapy; O = ovariectomy.

TABLE 3.—TSH levels before and during 5-FU treatment in 8 patients with advanced breast cancer

Patient No.	Previous treatment*	TSH level before treatment (microunits/ml)	TSH level (microunits/ml) during 5-FU treatment at Week—					
			4	8	12	16	20	> 20 (mean values)
2	M, R	6.9	9.7	4.9	10.2	8.7	10.0	—
3	M, R	8.8	7.4	6.4	—	—	—	—
8	M, O	10.0	—	7.4	9.7	13.4	5.3	7.9
9	R	5.1	4.7	7.7	4.8	5.0	7.4	7.7
10	M, R	10.9	5.1	6.5	11.2	8.4	8.7	7.9
11	M, R	11.0	7.8	11.9	12.3	13.6	10.9	—
13	O	5.9	6.1	9.3	4.3	9.3	6.7	—
15	R	5.2	5.1	8.6	6.8	5.0	6.1	—
Mean TSH		8.0	6.6	7.8	8.5	9.1	7.9	7.8
± SD		2.5	1.8	2.1	3.2	3.5	2.1	0.1

*M = mastectomy; R = radiotherapy; O = ovariectomy.

TT_4 in 17 courses of treatment in 15 patients significantly increased ($P^{xx} < 0.001$) from 9.2 ± 1.4 to 13.6 ± 3.3 μg/100 ml after 12 weeks of therapy and thereafter plateaued at about this level during further treatment (table 1). Already within 4 weeks of 5-FU treatment, the TT_4 rise was significant ($P^{x} < 0.01$). The relative increment of the mean TT_4 was about 50%. This TT_4 rise was observed in all of the 15 patients.

Table 2 documents the effect of 5-FU treatment on TT_3 levels in nine of these patients. In four patients, TT_3 duplicate pretreatment values closely agreed. The mean TT_3 level in this group was also found to rise significantly from 152 ± 35 ng/100 ml before treatment, to 206 ± 51 ng/100 ml within 12 weeks of treatment ($P^{xx} < 0.02$). In this period the relative increment of the mean was about 30%. After stopping 5-FU therapy in three of these patients, both TT_4 and TT_3 levels reached pretreatment values within 4 weeks (mean TT_4 and TT_3, respectively, 8.6 ± 1.8 μg/100 ml and 130 ± 30 ng/100 ml before treatment, and 8.5 ± 2.1 μg/100 ml and 136 ± 15 ng/100 ml after stopping treatment).

Table 3 illustrates that TSH levels did not change significantly during 5-FU treatment in eight patients studied and, in fact, remained measurable. The changes of the TSH levels in this group of patients, though apparently large in individuals, were not significant ($P^{xx} > 0.2$).

Table 4 documents that both before and during 5-FU treatment, AfT_4 levels were closely similar. The TT_4 levels during treatment ranged from 8.0 to 15.0 μg/100 ml, with AfT_4 values calculated thereafter ranging from 1.12 to 2.30 ng/100 ml.

It is emphasized that the patients did not complain of symptoms and none had clinical signs of hyperthyroidism.

TABLE 4.—TT_4, fT_4%, and AfT_4 before and during 5-FU treatment

Patient No.	Serum levels— Before treatment with 5-FU: TT_4 (μg/100 ml)	fT_4 (%)	AfT_4 (ng/100 ml)	During treatment with 5-FU: TT_4 (μg/100 ml)	fT_4 (%)	AfT_4 (ng/100 ml)
4	10.3	0.020	2.06	12.8	0.012	1.54
				15.0	0.013	1.96
6	9.7	0.021	2.06	11.1	0.019	2.09
				11.9	0.015	1.79
7	7.7	0.019	1.44	8.0	0.014	1.12
5	—	—	—	13.6	0.014	1.89
16	—	—	—	13.0	0.013	1.69
				14.2	0.016	2.27
17	—	—	—	12.9	0.013	1.68
				13.0	0.013	1.69
18	—	—	—	13.5	0.017	2.30

DISCUSSION

TT_4 and TT_3 levels of postmenopausal or ovariectomized women with metastatic breast cancer obviously increased during treatment with 5-FU. AfT_4 concentrations during such treatment did not differ to an appreciable extent from the AfT_4 values found in the euthyroid controls. Serum TSH levels before treatment were measurable, in contrast to the undetectably low values found in most patients with overt hyperthyroidism, and remained unchanged during repeated 5-FU treatments. In accordance with these laboratory data, no clinical signs of hyperthyroidism occurred. In the individual patients, the relative change in TT_4 and TT_3 was quite variable. Our data do not allow us to plausibly explain this variability.

Cross-sectional analysis of TT_4, TT_3, and TSH levels in 146 patients with breast cancer, primary and metastatic, did not disclose any systematic change in the various stages and did not differ from the corresponding values in the group of patients reported in this study (Beex L, and Ross A, unpublished observations). It is well known that in acute or chronic illness, TT_3 values may be subnormal and may return to normal at recovery. In this group of patients, however, TT_3 levels rose from normal to supranormal, whereas the patients were in a clinically stable condition. Recently it was reported by Levy et al (5) that physical illness in 15% of hospitalized patients might induce elevated serum TT_4 levels without concomitant rises of serum TT_3 levels. In the present study, however, both TT_4 and TT_3 levels increased in these nonhospitalized patients during repeated treatment with 5-FU. During the observation period, no patient was contaminated with radiographic dyes, excluding these agents as the cause of changes in serum TT_4 and TT_3 levels, as described by Bürgi et al (6). It seems inevitable to conclude that the observed effects are induced by the administration of the antimetabolite 5-FU itself.

The effect of 5-FU treatment on TT_4 and TT_3 levels in patients with other malignancies has not been studied systematically so far, although in two male patients with advanced adenocarcinoma receiving 5-FU treatment similar effects have been observed.

In earlier reports Dumont et al (7) and Curti et al (8) found a stimulating effect of 5-FU on thyroidal ^{131}I-uptake in mice and rats. However, Rodesch et al (9) and Vagenakis et al (10) suggested that this effect was primarily extrathyroidal in origin, ie, caused by iodine deficiency in the investigated animals. In a clinical study, Blomgren and Ansfield (11) evaluated the thyroid status in 13 patients following long-term treatment with fluoropyrimidines. In this study, normal to subnormal values for thyroidal ^{131}I-uptake were found. In agreement with these observations we did not find differences in the thyroidal ^{131}I-uptake in two of the patients before and during 5-FU treatment (3-hour uptake, 11% and 18% before treatment and 12% and 17% after 16 weeks of 5-FU treatment; 24-hour uptake, 27% and 36% before treatment and 27% and 32% after 16 weeks of 5-FU treatment, patients 6 and 15, respectively). Thus, it is highly improbable that the reported increases of TT_3 and TT_4 during long-term 5-FU treatment are a consequence of altered thyroid function.

It is unlikely as well that the observed effects are due to interference of 5-FU or its metabolites with the hormone assay procedure. First, 5-FU disappears from the plasma after iv injection within hours (12). Furthermore, a direct effect of 5-FU on the assay procedures was excluded.

The observed effects of long-term 5-FU treatment on TT_4 and TT_3 levels resemble the changes of thyroid hormone concentrations during pregnancy or during treatment with estrogens. Indeed, Kuo et al (13) observed a marked increase of total plasma estrone and estradiol levels in rats during treatment with high doses of 5-FU without signs of hyperestrogenicity.

The reported increase of TT_3 and TT_4 levels might therefore be interpreted to mean that long-term 5-FU treatment induces an increase of total binding capacity of the serum for thyroid hormones. The data in this study do not allow us to suggest if such increased binding of thyroid hormones of the serum is due to increased levels of any of the binding proteins (either of the prealbumin, the albumin, the specific thyroid binding globulin, or the immunoglobulin type) or to a qualitative change in their binding capacity.

As a final comment, patients with malignant diseases are often treated with extensive chemotherapy, sometimes even leading to further deterioration in well being. In patients receiving 5-FU treatment, clinicians unaware of this drug's associated increase of TT_4 and TT_3 might be tempted to prescribe unnecessary antithyroid treatment for otherwise euthyroid patients.

REFERENCES

1. MURPHY BEP, and JACHAN L. The determination of thyroxine by competitive protein binding analysis employing an anion-exchange resin and radiothyroxine. Lab Clin Med 66:161–167, 1965.
2. GHARIB H, RYAN RJ, MAYBERRY WE, ET AL. Radioimmunoassay for triiodothyronine (T_3). I. Affinity and specificity of the antibody for T_3. J Clin Endocrinol Metab 33:509–516, 1971.
3. HALL R. The immuno-assay of thyroid-stimulating hormone and its clinical applications. Clin Endocrinol 1:115–125, 1972.
4. HERRMANN J, and KRÜSKEMPER HL. Freies thyroxin im Serum. Zeitschrift Klin Chem Klin Biochem 9:320–323, 1971.
5. LEVY RP, LAUS VG, and HAMLIN CR. Hyperthyroxinemia and hypothyroxinemia in hospitalized patients. (Abstr No. 630) *In* Proceedings of the 58th Annual Meeting of the Endocrine Society, San Francisco, 1976.
6. BÜRGI H, WIMPFHEIMER C, BURGER A, ET AL. Changes of circulating thyroxine, triiodothyronine and reverse triiodothyronine after radiographic contrast agents. J Clin Endocrinol Metab 43:1203–1210, 1976.
7. DUMONT JE, RODESCH FR, and ROCMANS P. Stimulation of thyroidal radioiodine uptake by actinomycin and fluorouracil. Biochem Pharmacol 13:935–937, 1964.
8. CURTI JT, RUPP JJ, and CANTAROW A. Influence of 5-fluorouracil on thyroid function. Proc Soc Exp Biol Med 125:1125–1126, 1967.
9. RODESCH F, ROCMANS P, and DUMONT JE. Stimulation of thyroidal radioiodine uptake by actinomycin D and fluorouracil—an indirect effect. Biochem Pharmacol 16:907–908, 1967.
10. VAGENAKIS AG, BRAVERMAN LE, FOSTER AE, ET AL. Stimulatory effect of 5-fluorouracil on thyroid/serum iodide concentration ratios in the rat. Endocrinology 88:1250–1252, 1971.
11. BLOMGREN SE, and ANSFIELD FJ. Thyroid status following prolonged therapy with fluoropyrimidines. JAMA 193:51, 1965.
12. DOUGLASS HO, JR, and MITTELMAN A. Metabolic studies of 5-fluorouracil—II. Influence of the route of administration on the dynamics of distribution in man. Cancer 34:1878–1881, 1974.
13. KUO EYH, ESBER HJ, TAYLOR DJ, ET AL. Effects of cancer chemotherapeutic agents on endocrine organs and serum levels of estrogens, progesterone, prolactin and luteinizing hormone. Cancer Res 35:1975–1980, 1975.

Localization of *meso*-Tetra(*p*-sulfophenyl)porphine in Murine Sarcoma Virus-Induced Tumor-Bearing Mice[1,2,3]

Carl J. Carrano, Minoru Tsutsui,* and Stewart McConnell[4]

SUMMARY

***meso*-Tetra(*p*-sulfophenyl)porphine ($TPPS_4$) has been found to accumulate in the tumors of mice bearing a murine sarcoma virus-induced rhabdomyosarcoma to a greater degree than in all other tissues except for the kidney. Tumor to tissue ratios of from 3:1 (tumor to liver) to 9:1 (tumor to muscle) were observed. The uptake of $TPPS_4$ was found to be dose dependent, and the highest tumor to tissue ratios were found 96 hours after injection. The water-soluble $TPPS_4$ appears to be cleared through the kidneys and absolute concentrations there are reduced with increasing time after injection. These data are compared with previous studies on $TPPS_4$ localization in Walker 256 carcinosarcoma-bearing mice. In view of this localizing ability and its relative lack of toxicity, $TPPS_4$ seems to represent an ideal candidate for a tumor-localizing agent if high levels of the porphyrin in the kidneys can be reversed.**

[Cancer Treat Rep 61:1297–1300, 1977]

Since the observation in 1924 that porphyrins caused red fluorescence in tumors but not other tissues of experimental animals, much work on porphyrins as tumor localizers has appeared. (For a recent review see Tsutsui et al [1].) A significant advance in this area was the introduction by Winkelman et al (2) of the synthetic porphyrin, tetraphenylporphine sulfonate (TPPS).[5] Using this compound in rats bearing a Walker 256 carcinosarcoma, Winkelman was able to demonstrate that the TPPS concentrated exclusively in tumors relative to other tissues and organs.

This study remained one of the few quantitative examinations of porphyrin localization in tumors. It was desirable to see if the reported localizing ability of TPPS could be extended to other tumor systems. Accordingly, this study was initiated using highly purified *meso*-tetra(*p*-sulfophenyl)porphine ($TPPS_4$) (fig 1) (completely tetra sulfonated) and a virus-induced tumor in mice.

EXPERIMENTAL METHODS

Toxicity

Female BALB/cTex mice, obtained from Texas Inbred Mice Co (Houston), were injected ip with $TPPS_4$ dissolved in physiologic saline. The injected dose corresponded to 50–250 mg/kg of $TPPS_4$ per 20 g of animal. The animals were returned to their cages and observed for toxic effects for 1 week.

Localization Studies

Murine sarcoma virus (MSV) was used to induce a rhabdomyosarcoma in the hind legs of BALB/cTex mice. The MSV preparation was received as a frozen tumor extract from Mr. Galen Marshall, University of Texas Medical Branch, Galveston. Each animal was injected im with 0.1 ml of the extract into the hind legs. After approximately 12 days the tumor had reached maximum growth and localization experiments were begun. A physiologic saline solution containing 1.5–3.0 mg of $TPPS_4$ was

[1]Received Nov 26, 1976; revised Apr 4, 1977; accepted Apr 20, 1977.

[2]Supported by National Institutes of Health Biomedical grant 55142, Texas A and M University Health Fellowship (C. J. Carrano), and Robert A. Welch Foundation grant A-420.

[3]*meso*-Tetra(*p*-sulfophenyl)porphine: NSC-177391.

[4]Departments of Chemistry (C. J. Carrano and M. Tsutsui) and Veterinary Microbiology (S. McConnell), Texas A and M University, College Station.

[5]TPPS refers to an unpurified mixture of isomers.

**Reprint requests to:* Dr. Minoru Tsutsui, Department of Chemistry, Texas A and M University, College Station, Tex 77840.

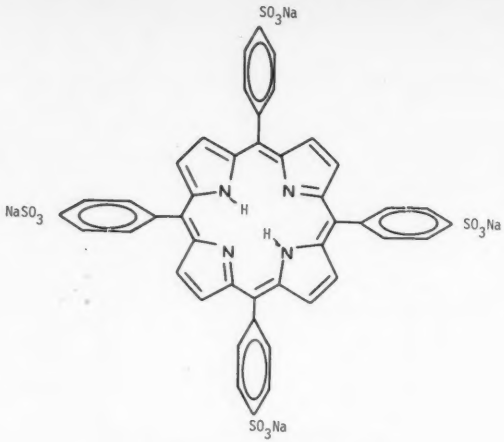


FIGURE 1.—Structure of $TPPS_4$.

injected either ip or iv (tail vein) into each animal. Replicate numbers of treated and untreated mice were killed on Days 1–4 by ether anesthesia. Experiments were terminated after 96 hours due to spontaneous regression of the tumors at longer times. The various organs were rapidly excised and frozen until ready for biochemical processing. $TPPS_4$ was extracted by a modification of the method of Winkelman (3). Briefly, the tissues were homogenized in 0.25 M sucrose to give a homogenate containing 0.1 g/ml. One ml of this was diluted to 2 ml with water and 2 ml of 10% trichloroacetic acid (TCA) was added. Winkelman states that all the $TPPS_4$ present precipitates with the protein; we did not find this to be the case. In our studies approximately 20% of the $TPPS_4$ remained in solution. This $TPPS_4$ was measured directly at 440 nm using a standard curve constructed by adding known quantities of $TPPS_4$ to a 10% solution of TCA. The precipitate which contained the bulk of the porphyrin was stirred with 5 ml of methanol:NH_4OH (10:1) overnight and centrifuged. The $TPPS_4$ was solubilized by this procedure. One ml of this solution was brought to a volume of 5 ml by the addition of methanol/water solution (pH 2). The $TPPS_4$, now in its acid form, was measured at 440 nm.

$TPPS_4$ was synthesized by the method of Srivastava and Tsutsui (4) and purified by chromatography on Sephadex LH-20.

RESULTS

Acute Toxicity In Vivo

BALB/cTex mice were used to determine the acute toxicity of injected $TPPS_4$. Doses ranging from 50 to 250 mg/kg of $TPPS_4$ were injected ip and the mice were examined for toxic reactions. Mice receiving 250 mg/kg of $TPPS_4$ in 0.5 ml of physiologic saline showed evidence of discomfort immediately after injection. Muscular weakness and general malaise appeared to be the only observable signs. Within several hours the mice appeared to be completely recovered. No deaths occurred at any of the dose levels after 1 week of observation, nor was there any obvious pathology present. Thus, doses of up to 250 mg/kg of $TPPS_4$ appear to be tolerated by mice.

In Vivo Localization

Tests on $TPPS_4$ localization in mice bearing MSV-induced rhabdomyosarcomas reveal significant differences from Winkelman's study of rats with the Walker 256 carcinosarcoma. $TPPS_4$ is found to concentrate in tumors relative to other tissues except for levels in the kidney which have values consistently greater than that of the tumors. The tumor to tissue ratios observed are also not as good as those found by Winkelman. Table 1 shows the concentration of $TPPS_4$ in various organs of BALB/cTex mice at various doses of porphyrin. The tissue concentrations are roughly proportional to the injected dose. The liver and lung generally contain about the same quantity of porphyrin with less porphyrin observed in other tissues. The kidney always has a higher concentration of $TPPS_4$ than the tumor or any other organ. The $TPPS_4$ concentration in the tumor is quite variable;[6] this is probably due to the nature of the tumor itself. The rhabdomyosarcoma is an invasive malignancy of the muscle. As a consequence it is difficult, in fact impossible, to obtain the tumor free from muscle and bone. Since muscle possesses one of the lowest concentrations of $TPPS_4$, as demonstrated by Winkelman and confirmed by us, the reported concentration in the tumor is most probably erroneously low. Tumor to liver ratios of from 5:1 to 2:1 are observed, with all other tumor to tissue ratios even higher, except the tumor to kidney ratios which range from 1:5 to 1:2. Table 2 shows the effect of time after injection on localization. Increasing the time, up to at least 96 hours when the experiment was terminated, results in better localization (ie, higher tumor to tissue ratios). The $TPPS_4$ concentrations in the tumor and liver remain fairly constant while the $TPPS_4$ concentration in the kidney is reduced. This is not unexpected, because the water-soluble $TPPS_4$ is initially cleared through the

[6]The average deviation for tumor values in tables 1 and 2 is ±10% while the deviation for the other organs is ≤±4%.

TABLE 1.—Content of $TPPS_4$ in tissues of tumor-bearing mice at various doses of porphyrin after 48 hrs (μg $TPPS_4$/0.1 g tissue, wet weight)

Tissue	Dose (mg/kg)*		
	250	200	100
Tumor	64.7	21.0	24.8
Liver	22.9	11.2	5.1
Lung	22.5	11.7	3.1
Kidney	127.4	92.2	43.1
Heart	15.7	ND	5.0
Muscle	7.5	ND	ND
Spleen	ND	12.8	ND

*ND = not determined.

TABLE 2.—Content of $TPPS_4$ in tissues of tumor-bearing mice at various times after injection of $TPPS_4$ (μg $TPPS_4$/0.1 g tissue, wet weight)*

Tissue	Content in tissues after 3 mg of $TPPS_4$	
	24 hrs	72 hrs
Tumor	16.7	17.4
Kidney	72.3 (0.24)	42.4 (0.42)
Liver	14.2 (1.2)	8.1 (2.2)
	Content in tissues after 4 mg of $TPPS_4$	
	72 hrs	96 hrs
Tumor	27.4	34.4
Kidney	79.5 (0.34)	70.7 (0.49)
Liver	10.4 (2.6)	11.1 (3.1)

*Nos. in parentheses = tumor to tissue ratios.

kidneys. Little or no differences were found in the pattern of localization with the mode of injection. Either ip or iv injections give similar results.

DISCUSSION

To date, the only study of localization of $TPPS_4$ in tumor-bearing animals has been that of Winkelman on rats bearing the Walker 256 carcinosarcoma. Studies on other tumor types in a variety of laboratory animals were clearly lacking. Using MSV-induced rhabdomyosarcomas of BALB/cTex mice we have examined the ability of $TPPS_4$ to localize in this tumor. In contrast to Winkelman's studies, we find that the highest concentrations of porphyrin after injection are found in the kidneys in all animals tested. This is true regardless of the time of sampling or of the amount of the injected dose. Levels in the tumors are, however, quite high and indeed exceed that of any other tissue measured except the kidney. Tumor to tissue ratios of from 2:1 to 5:1 are observed for the liver and are even greater for all other organs. These values are probably very conservative because $TPPS_4$ concentration in pure tumor tissue uncontaminated by muscle would undoubtedly have been higher.

Winkelman found similar tumor to tissue ratios but only small quantities of porphyrin were found in the kidneys. We found higher absolute concentrations of $TPPS_4$ in all tissues assayed than did Winkelman.

The best tumor to tissue ratios were achieved with relatively high doses of porphyrin and for long times after injection. The porphyrin content of the tumor is seen to rise with increasing time following injection while its content in the kidney drops over the same span.

Recent work by Hambright et al (5) has shown that metalloporphyrins, including ^{109}Pd-$TPPS_4$, do not localize well in a variety of tumors. These results indicate little specificity of uptake on tumor type or on porphyrin side chains. Winkelman has also shown that metallo-derivatives of $TPPS_4$ lose most of their localizing ability.

It is clear that the chelation of the metal ion destroys tumor-localizing ability for all porphyrins tested. However, we have confirmed that the free-base $TPPS_4$ is indeed concentrated in tumors relative to other tissue. The addition of metal ions to the central "hole" of the porphyrin macrocycle profoundly changes many of its physical-chemical properties. Differences in charge distribution caused by d_z2 orbitals in transition metal ions coordinated to the porphyrin could change the ability to bind to cellular receptors. Transport may also be affected by the physical state in solution. $TPPS_4$ normally appears as a dimer (6) in aqueous solutions while its metallo-derivatives are monomeric. The central nitrogen atoms may play a key role in localization or transport and these are tied up upon chelation. In any event, the use of metallo-$TPPS_4$ derivatives for therapeutic or diagnostic delivery of radioactive isotopes to neoplastic tissue is not encouraging. The reconfirmation of the localizing ability of the free base, however, does provide a measure of hope for preparing effective diagnostic and chemotherapeutic derivatives. A number of alternatives which may not have such an adverse effect on localizing ability of the parent porphyrin are possible.

In addition, $TPPS_4$ achieves much higher absolute tumor concentrations than hematoporphyrin. The latter has achieved some success as a photosensitizer of tumor tissue (7). Photodynamic therapy might be improved by the use of $TPPS_4$. These concepts are currently being tested in this laboratory.

In summary, $TPPS_4$ has been demonstrated to concentrate in tumors relative to tissue with the exception of higher levels in the kidney. These high

levels might be reduced by the stimulation of excretion via diuretics. The possible diagnostic or therapeutic use of $TPPS_4$ must take into account the extra-tumor distribution and high kidney levels observed in this and other studies.

REFERENCES

1. TSUTSUI M, CARRANO CJ, and TSUTSUI EA. Tumor localizers: porphyrins and related compounds (unusual metalloporphyrins XIII). Ann NY Acad Sci 244:674–684, 1975.
2. WINKELMAN J, SLATER G, and GROSSMAN J. The concentration in tumor and other tissues of parenterally administered tritium and C-14 labeled tetraphenylporphine-sulfonate. Cancer Res 27:2060–2064, 1967.
3. WINKELMAN J. The distribution of tetraphenylporphine-sulfonate in the tumor bearing rat. Cancer Res 22:589–596, 1962.
4. SRIVASTAVA TS, and TSUTSUI M. Preparation and purification of tetrasodium *meso*-tetra(*p*-sulfophenyl) porphine, an easy procedure. J Org Chem 38:2103, 1973.
5. HAMBRIGHT P, RAWWAZ R, VALK P, ET AL. The distribution of various water soluble radioactive metalloporphyrins in tumor bearing mice. Bioinorg Chem 5:87–92, 1975.
6. HAMBRIGHT P. Dynamic coordination chemistry of metalloporphyrins. *In* Porphyrins and Metalloporphyrins (Smith KM, ed). New York, American Elsevier Publishing Co, 1975, chap 6.
7. DOUGHERTY TM. Photo-radiation of malignant tumors. *In* Abstracts of Papers Presented at the 100th American Chemical Society National Meeting, Chicago, Ill, Medi-014, 1973.

Evaluation of Radiotherapy for Localized Inflammatory Skin and Perianal Lesions in Adult Leukemia: A Prospectively Randomized Double Blind Study [1]

John A. Levi,[2] Stephen C. Schimpff,[2]* Robert G. Slawson,[3] and Peter H. Wiernik [2]

SUMMARY

Thirty-five episodes of localized infection among 20 patients with acute leukemia and granulocytopenia (< 1000 cells/μl) were treated with intensive supportive care including systemic antibiotics. In a double blind randomization, 17 of the episodes also received 400 rads of megavoltage irradiation to the site of the lesion given as a single dose and repeated 7 days later. No significant differences were observed in response rates between the irradiated and nonirradiated lesions. Overall, 65% of the irradiated infections and 44% of the nonirradiated lesions responded completely, a difference which is not significant. The median number of days required to achieve maximum clinical response was similar whether or not irradiation was given (10 versus 12 days). Management of such localized infections should include early diagnosis with prompt institution of intensive supportive care including appropriate systemic antibiotics, but low-dose irradiation cannot be recommended as part of the routine management of these lesions.

[Cancer Treat Rep 61:1301–1305, 1977]

Primary skin infections are an important source from which disseminated sepsis may arise in granulocytopenic cancer patients. Perianal and axillary lesions are common in patients with acute nonlymphocytic leukemia and carry a poor prognosis (1–6). One suggestion for the management of anorectal lesions has been the use of low-dose megavoltage irradiation in conjunction with systemic antibiotics (2). The present study was therefore undertaken to evaluate in a prospective, randomized fashion whether low-dose megavoltage radiation has any role in facilitating healing and preventing dissemination of localized primary skin lesions in granulocytopenic patients with leukemia.

METHODS

Eligible patients were those adults with acute leukemia, a granulocyte count of < 1000/μl, and a new localized inflammatory lesion not secondary to disseminated infection. Pretreatment evaluation included measurement of the degree of erythema, induration, and, if present, fluctuation and drainage. Needle aspirations were obtained whenever possible for an appropriate gram stain and culture in addition to swab cultures of the lesion plus aerobic and anaerobic blood cultures.

All patients received intensive supportive care which included: warm peroxide compresses three times daily, local application of a triple antibiotic cream (neomycin, bacitracin, and polymyxin) three times daily, analgesics when indicated for pain, and low-bulk diets, stool softeners, or antidiarrheal agents as appropriate for anorectal lesions. When fever was present, patients were empirically begun on systemic antibiotics (a two-drug combination of carbenicillin [or ticarcillin], gentamicin, and cephalothin) (7). Antibiotics were subsequently altered, if necessary, according to the results of cultures. Granulocyte transfusions were not given unless the lesion was clearly progressing indicating that the initial therapy had failed (8). Surgery was only performed when there was a definite area of fluctuation present and consisted of the smallest possible procedure necessary to allow adequate drainage (1).

[1]Received Dec 3, 1976; revised May 24, 1977; accepted May 25, 1977.

[2]Baltimore Cancer Research Center (BCRC), Division of Cancer Treatment, National Cancer Institute, Md.

[3]Department of Radiotherapy, University of Maryland Hospital, Baltimore.

Reprint requests to: Stephen C. Schimpff, MD, BCRC, University of Maryland Hospital, 22 S Greene St, Baltimore, Md 21201.

TABLE 1.—Patient characteristics, sites of lesions, and pathogens

	Irradiation (17 lesions)	No irradiation (18 lesions)
Leukemia diagnosis		
Acute myelomonocytic leukemia	10	12
Acute myelocytic leukemia	4	5
Chronic myelocytic leukemia with blastic transformation	2	0
Acute lymphocytic leukemia	1	1
Initial granulocyte count (cells/μl)		
0–99	8	8
100–499	6	8
500–999	3	2
Mean, range	251, 0–900	263, 5–999
Site of lesion (microbiologic documentation)		
Perianum	7 (4)	5 (3)
Axilla	6 (1)	7 (5)
Other skin sites	4 (3)	6 (3)
Totals	17 (8)	18 (11)
Pathogens (bacteremias)		
Perianum	*Pseudomonas aeruginosa*, 1 *Klebsiella pneumoniae*, 2 *Escherichia coli* and *Klebsiella pneumoniae*, 1 (1)	*Pseudomonas aeruginosa*, 2 (1) *Proteus mirabilis* and *Escherichia coli*, 1
Axilla	*Escherichia coli* and *Serratia marcescens*, 1 (1)	*Klebsiella pneumoniae*, 1 *Proteus mirabilis* and *Staphylococcus aureus*, 3 *Pseudomonas aeruginosa* and *Staphylococcus aureus*, 1
Other skin sites	*Pseudomonas aeruginosa*, 1 *Staphylococcus aureus*, 1 *Proteus mirabilis* and *Staphylococcus aureus*, 1	*Pseudomonas aeruginosa*, 1 (1) *Escherichia coli*, 1 *Staphylococcus aureus*, 1

After obtaining informed consent, the patients were referred to the radiotherapist who randomly assigned the patient to receive or not receive irradiation. The sealed randomization cards were stratified for perianal, axillary, or skin lesions at other sites. Patients randomized to receive radiotherapy were given 400 rads of megavoltage irradiation as a single treatment to the site of the lesion using a port large enough to cover the lesion with adequate margins. This course was repeated 7 days later, irrespective of the immediate results, bringing the total dose of irradiation delivered to 800 rads. All patients, including those randomized not to get irradiation, were placed under the radiation unit on both dates. Neither the patient nor the medical staff were aware of the randomization result until after evaluation was completed.

Following the initiation of therapy, daily flow sheets were independently kept by both the primary care physician and the principal investigators. Assessment included subjective changes in pain, tenderness, and general performance status, and objective determinations of fever, area of induration, and erythema plus the degree of fluctuation and drainage, if present. Granulocyte and platelet counts were obtained at least twice weekly.

Evaluation of the lesions was continued for a 21-day period. Responses were classified as: (*a*) *complete*—complete loss of pain and tenderness, disappearance of fever, induration, and erythema, or spontaneous drainage and healing; (*b*) *partial*—substantial decrease (> 50%) in the objective parameters of the lesion but less than complete response within the 21-day period; and (*c*) *progression*—any continued increase in the area of induration and erythema within 7 days after the initiation of therapy irrespective of subsequent changes over the 21-day observation period. Following the completion of the study, all previously involved sites were checked regularly for any signs of recurrence.

RESULTS

Patient Characteristics

Twenty adult patients with acute leukemia had 35 separate episodes of primary localized skin or perianal infections (table 1). The 17 irradiated and 18 nonirradiated patient trials were equivalent with respect to leukemia type and therapy and initial absolute granulocyte count.

Sites of Lesions and Microbiologic Documentation

The 17 irradiated and 18 nonirradiated lesions were comparable with regard to number in the axilla, perianum, or other skin sites (table 1). The frequency of microbiologic documentation was equivalent for the irradiated and nonirradiated perianal and other skin site lesions. However, only one of six irradiated axillary lesions compared to five of seven nonirradiated axillary lesions was microbiologically documented suggesting the possibility that by chance allocation, the nonirradiated axillary lesions might have been more severe at the time of therapy initiation.

Twenty of 27 isolated pathogens were gram-negative bacilli with the remaining seven being *Staphylococcus aureus* (table 1). Bacteremia occurred in two irradiated and two nonirradiated patients.

Response to Therapy

There were no significant differences between the irradiated and nonirradiated groups with regard to the percentage of infections which resolved completely or the median days to complete response (table 2). Although only one of seven of the nonirradiated axillary lesions (14%) responded completely

TABLE 2.—Sites of infection and response to therapy

	Infection sites							
	Perianum		Axilla		Other skin		Total	
	Irradiation	No irradiation	Irradiation	No irradiation	Irradiation	No irradiation	Irradiation	No irradiation
Response to therapy, all lesions								
Complete	5 (71%)	3 (60%)	3 (50%)*	1 (14%)*	3 (75%)	4 (67%)	11 (65%)	8 (44%)
Partial	2 (29%)	1 (20%)	1 (17%)	4 (57%)	1 (25%)	0 (0)	4 (24%)	5 (27%)
Progression	0 (0)	1 (20%)	2 (33%)	2 (28%)	0 (0)	2 (33%)	2 (12%)	5 (27%)
Median days to complete response (range)	11 (6–15)	12 (6–15)	8 (6–10)	10	10 (8–10)	10 (4–21)	10 (6–15)	12 (4–21)

*P = 0.217, Fisher exact test, 2-tailed analysis.

TABLE 3.—Response to therapy according to rise in granulocyte count

Rise in granulocyte count (cells/μl)*	Response to therapy			
	Complete	Partial	Progression	Totals
Irradiation				
< 100	9†	2	2	13
100–500	1	0	0	1
> 500	1	2	0	3
No irradiation				
< 100	3†	4	2	9
100–500	1	1	1	3
> 500	4	0	2	6

*Change in granulocyte count assessed over a 21-day period from start of infection for the responding lesions.

†$P = 0.11$, Fisher exact test, 2-tailed analysis.

compared to three of six irradiated axillary lesions (50%), the difference is not significant ($P = 0.22$).

Five of the seven infections which progressed, at any site, were microbiologically documented and were caused, in part, by gram-negative bacilli. Three of the four lesions with associated bacteremia progressed (irradiated, one of two lesions; nonirradiated, two of two).

Granulocyte Count and Therapeutic Response

The absolute granulocyte count failed to rise above 100/μl during therapy in 13 of the 17 infections in the irradiated group and in nine of the 18 infections in the nonirradiated group during the 21-day assessment period (table 3). Nine of the 13 irradiated lesions (69%) and three of the nine nonirradiated lesions (33%) completely responded despite persistent granulocytopenia ($P = 0.11$). However, this possible trend in favor of the irradiated lesions does not pertain if only microbiologically documented infections are considered.

DISCUSSION

Localized inflammatory lesions in patients with acute leukemia and associated granulocytopenia tend to present with an atypical clinical picture (1,5,9). The lesions most often appear as indurated, tender areas; progressively, the surrounding tissue will become erythematous and more indurated (cellulitis) but fluctuation (abscess) is not usually prominent (1,9). Awareness of such an atypical picture is important to avoid any delays in diagnosis with the attendant risks of disseminated sepsis developing. The present study has shown that generally good results may be achieved when the lesions are diagnosed early and when intensive supportive care, including the prompt use of broad spectrum bactericidal systemic antibiotics, is begun. In this series, all patients were treated promptly, received the same or similar antibiotic regimens, and were given granulocyte transfusions in the event of infection progression. Response criteria were stringently followed; progression during the first week followed by improvement later was defined as progression.

We were unable to confirm the suggestion of Sehdev et al (2) that the addition of low-dose megavoltage irradiation had any significant influence on the overall outcome of these infections. Dermatologists have used low-dose irradiation for the treatment of acne vulgaris and, although this form of treatment has generally fallen into disfavor, successful results were claimed when the acne was severe and mutilating (10). Several investigators have also reported consistently good results for suppurativa hidradenitis of the axilla with irradiation therapy (11,12). These infections may be similar in origin to the axillary lesions observed in the present study and are consistent with the possibility that irradiation may be of some benefit for axillary lesions in patients with acute leukemia. Nevertheless, our results from this double blind trial do not justify the routine use of low-dose irradiation for such infections.

Gram-negative organisms as a group accounted for most isolates from the lesions in the present series including the axilla (six of 13 lesions); this is consistent with previously reported data on such local infections (1–3, 8). *Staphylococcus aureus* either alone or with other organisms was the single most common organism isolated from axilla or other skin lesions but was not found in perianal lesions. These findings, especially the frequency of gram-negative infection, should be taken into account when selecting the initial parenteral antibiotic regimen prior to the isolation of any specific organism. Preinfection surveillance culture results from axilla or perianum may assist in this (6), since frequently organisms isolated from these areas were the organisms responsible for the subsequent infection.

Although most infections eventually responded to appropriate therapy, prevention is the key to improved patient comfort and reduced morbidity. Oral nonabsorbable antibiotics and efforts to reduce hospital acquisition of *Pseudomonas aeruginosa* will substantially decrease the incidence of perianal infections (13,14). Reduced acquisition of *Staphylococcus aureus* (eg, personnel handwashing) and attention to patient hygiene will decrease axillary and other skin infections. During the past 18 months we have utilized an intensive program of patient hygiene supervised by an infection control nurse in conjunction with the use of povidone-iodine swab sticks applied to the axilla four times daily. This program has essentially eliminated axillary lesions, the only occurrences being in two patients who discontinued use of the povidone-iodine swabs.

The present study would suggest that the principal factors required in the management of localized infective skin or perianal lesions in patients with acute leukemia include a high index of suspicion, early diagnosis, and prompt institution of intensive supportive care including systemic antibiotic therapy. The addition of low-dose irradiation to this therapeutic approach was not shown to be of benefit.

REFERENCES

1. SCHIMPFF SC, WIERNIK PH, and BLOCK JB. Rectal abscesses in cancer patients. Lancet 2:844–847, 1972.
2. SEHDEV MK, DOWLING MD, JR, SEAL SH, ET AL. Perianal and anorectal complications in leukemia. Cancer 31:149–152, 1973.
3. SPIERS ASD, TATTERSALL MHN, and GAYA H. Indications for systemic antibiotic prophylaxis in neutropenic patients. Br Med J 2:440–441, 1974.
4. DREIZEN S, BODEY GP, RODRIGUEZ V, ET AL. Cutaneous complications of cancer chemotherapy. Postgrad Med 58:150–158, 1975.
5. SCHIMPFF SC. Diagnosis of infection in patients with cancer. Eur J Cancer 11(Suppl):S29–S38, 1975.
6. SCHIMPFF SC, YOUNG VM, GREENE WH, ET AL. Origin of infection in acute nonlymphocytic leukemia: significance of hospital acquisition of potential pathogens. Ann Intern Med 77:707–714, 1972.
7. GAYA H, KLASTERSKY J, SCHIMPFF SC, ET AL. Protocol for an international prospective trial of initial therapy regimens in neutropenic patients with malignant disease. Eur J Cancer 11(Suppl):S1–S4, 1975.
8. SCHIFFER CA, AISNER J, BETTS SW, ET AL. Clinical experience with transfusion of granulocytes obtained by continuous flow filtration leukopheresis. Am J Med 58:373–381, 1975.
9. SICKLES EA, GREENE WH, and WIERNIK PH. Clinical presentation of infection in granulocytopenic patients. Arch Intern Med 135:715–719, 1975.
10. FRANK SB. Acne Vulgaris. Springfield, Ill, Charles C. Thomas, 1971, pp 294–310.
11. SCHENCK SG. Hidradenitis suppurativa axillaris. Radiology 54:74–77, 1950.
12. ZELIGMAN I. Temporary X-ray epilation therapy of chronic axillary hidradenitis suppurativa. Arch Dermatol 92:690–694, 1965.
13. LEVI JA, VINCENT PC, JENNIS F, ET AL. Prophylactic oral antibiotics in the management of acute leukemia. Med J Aust 1:1025–1029, 1973.
14. SCHIMPFF SC. Commentary: Laminar air flow room reverse isolation and microbial suppression to prevent infection in patients with cancer. Cancer Chemother Rep 59:1055–1060, 1975.

Tumor Size, Sensitivity to Therapy, and Design of Treatment Schedules [1]

Larry Norton* and Richard Simon [2]

A fundamental concept regarding the relationship between tumor size and sensitivity to therapy has for many years exerted profound influence on the design of cancer treatment protocols. This concept, stated simply, is that small tumors, due to their large fraction of actively dividing cells, are more "sensitive" to cytotoxic therapy than tumors of equivalent histology but larger size (1,2). So widely accepted is this belief that it has been incorporated, sometimes unintentionally, into treatment plans for both advanced inoperable tumors and tumors following surgical resection or de-bulking. In the treatment of advanced tumors, for example, many therapy schedules employ intensive therapy initially, when the tumor is largest, with tapering of dose levels as tumor bulk is reduced. If not by design, this schedule is often the result of dose modifications made necessary by the depletion of bone marrow reserve. Should radiotherapy or chemotherapy successfully achieve a clinical complete remission (CR), the dose levels of chemotherapy chosen to maintain the remission are often less intense than those employed to obtain the remission initially (3). In the post-surgical setting, where it is thought that only microscopic foci of tumor are left residual, the dose schedule of adjuvant chemotherapy chosen is often less intense than would be used for a larger tumor of equivalent type. This tendency to choose less intensive regimens for adjuvant use may be motivated not only by a desire to reduce acute toxicity, but also by an awareness of the possible long-term adverse consequences of chemotherapy, especially the increased risk of second malignancies (4–6).

In all of these cases, the less intensive treatment applied to the small tumor is thought sufficient on the belief that the tumor volume, reduced by the earlier application of intense therapy, is then more sensitive to treatment. Unfortunately, there are many clinical observations incompatible with this concept. For example, in the adjuvant therapy of operable breast cancer, comparatively low-dose and short-course regimens were employed in the past but were inferior in prolonging remission to the more intense protocols used in recent clinical trials (7–9). The application of 5-fluorouracil (5-FU) as a single agent, an effective therapy for regression of advanced colon carcinoma, has not proven effective as an adjuvant to surgery in operable disease (10). Similarly, the agent DTIC, which is of documented efficacy in the treatment of metastatic malignant melanoma (11), does not improve the disease-free interval when used as an adjuvant to surgery in disease involving regional nodes (12). These and many other observations strongly suggest that the microscopic foci left after surgical resection are sufficiently resistant to chemotherapy that they are not easily curable by low exposures. Another significant observation is that it is common for cases of Hodgkin's disease (13), Burkitt's lymphoma (14), acute lymphoblastic leukemia (15), and nodular non-Hodgkin's lymphoma, treated beyond the attainment of CR, to recur in the same site with retained sensitivity to the initial therapy. In many of these cases massive tumors may be reduced to microscopic disease by a relatively brief exposure to treatment, followed by prolonged therapy after CR is achieved. If the microscopic cancer left after the attainment of CR is then more sensitive to therapy, how does it survive treatment to regrow at a later time?

It seems, therefore, that the concept that sensitivity to therapy strictly increases with decreasing tumor size is not entirely in accord with clinical experience. On the other hand, it is an incontestable clinical impression that very large tumors are also usually therapy-resistant. Hence, clinical experience suggests that some tumors may be less sensitive to therapy when they are very small or very large than when they are of intermediate size. Clearly, a new view of the relationship between tumor size and sensitivity to therapy, one that is consistent with these clinical observations, would provide an attractive alternative to the currently accepted concept that "small is sensitive."

Such an alternative view is suggested by a close examination of the growth curves of tumors exposed to anticancer agents. The growth rates of most

[1]Received Dec 20, 1976; revised Apr 19, 1977; accepted May 2, 1977.

[2]Division of Cancer Treatment (DCT), National Cancer Institute (NCI), Bethesda, Md.

**Reprint requests to:* Larry Norton, MD, Department of Neoplastic Diseases, Mt. Sinai School of Medicine, 5th Ave and 100th St, New York, NY 10029.

untreated malignancies that have been evaluated (16) may be accurately described by the formula

$$dN(t)/dt = GF(N) \cdot N(t) \quad [1]$$

where dN(t)/dt is the growth rate, and N(t) is the tumor volume at time t. The symbol GF(N) stands for the "instantaneous growth fraction" or "specific growth rate" of a tumor of size N, and has a simple intuitive interpretation. In any small increment of time dt the tumor volume N(t) increases by a volume dN(t) which is equivalent to a fraction, given by the function GF(N), of the tumor volume N(t). The expression GF(N) may therefore be thought of as that fraction of N(t) which instantaneously doubles in size during dt. It is important to note that the empirical GF concept presented here is slightly different from that commonly employed by cell kineticists. As Steel has said (17), the growth fraction "is a term that has a precise meaning only in the context of a chosen mathematical model." The GF in our usage may not be strictly equal to the total number of cells in mitosis, but incorporates the cell loss associated with cell death or other removal processes that occur during unperturbed growth, as well as changes in tumor vascularity, hydration, and content of nonmalignant cells.

In the case of simple exponential growth, the GF(N) is by definition constant for all N. Writing GF(N) as a constant K_1, equation [1] may be written

$$dN(t)/dt = K_1 \cdot N(t). \quad [2]$$

While L1210 and other experimental tumors may approximate exponential growth, most experimental and clinical tumors do not (18), but are exponentially decremented with increasing size so that a limiting asymptotic size—a "plateau" size—is approached (fig 1, curve A). In this pattern of growth, termed "Gompertzian" after Benjamin Gompertz (19), GF(N) is not constant, but is proportional to the logarithm of the ratio $N(\infty)/N(t)$, where $N(\infty)$ is the ideal plateau size (16). In the Gompertzian case, equation [1] may therefore be written

$$dN(t)/dt = K_2 \cdot \ln \{N(\infty)/N(t)\} \cdot N(t), \quad [3]$$

where K_2 is a constant.

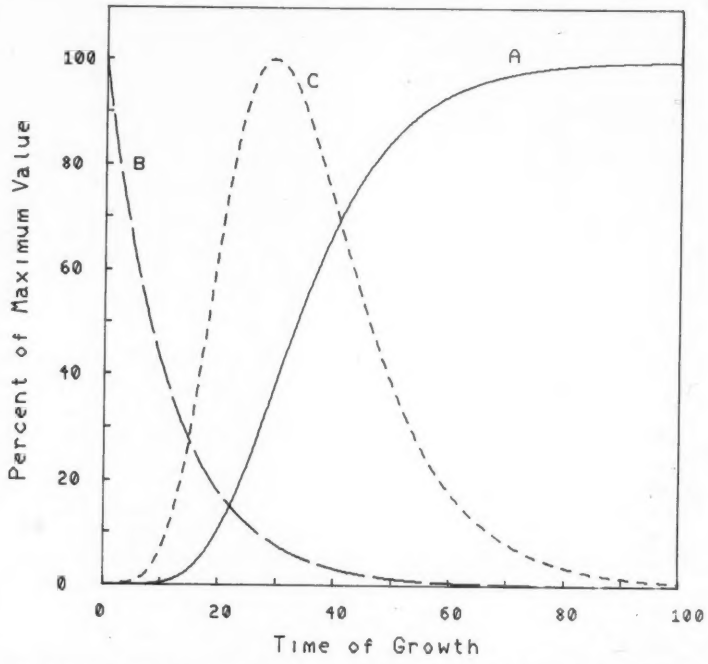
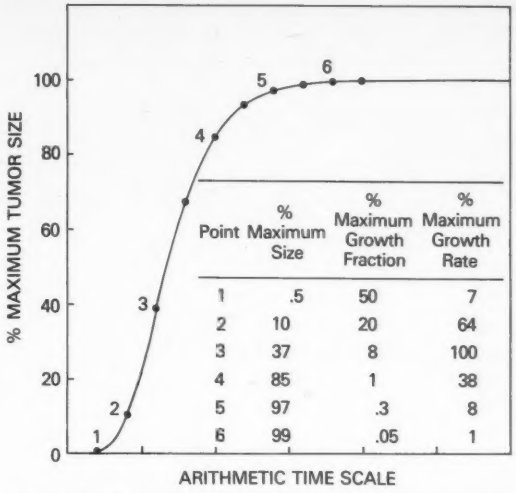


FIGURE 1.—Relationship between tumor size (curve A), instantaneous GF or specific growth rate (curve B), and growth rate (curve C) for unperturbed Gompertzian growth. While the GF is maximum at the time of initiation of growth, the growth rate is maximum when the tumor is about 37% of its limiting size. The Gompertzian equation used is

$$dN(t)/dt = B \cdot \log\{100/N(t)\} \cdot N(t)$$
$$B = 0.4/\log\{100\}$$



Point	% Maximum Size	% Maximum Growth Fraction	% Maximum Growth Rate
1	.5	50	7
2	10	20	64
3	37	8	100
4	85	1	38
5	97	.3	8
6	99	.05	1

FIGURE 2.—Relationship, for Gompertzian growth, between tumor size, instantaneous GF, and growth rate, presented in tabular form.

By inspection of equation [2], it is clear that for exponential growth, the growth rate increases in direct proportion to tumor size. For Gompertzian growth, however, the growth rate is smallest for both very small and very large tumors, and is maximum at a point called the "inflection point," when the tumor is about 37% of its maximum size (fig 1, curve C; fig 2, point 3). It is not generally realized that for Gompertzian growth, a large GF does not necessarily imply a large growth rate. The distinction between GF and growth rate is made clear in figures 1 and 2.

If a tumor is treated with effective therapy one would expect its growth rate to be decremented to a degree somehow dependent on the effectiveness of the anticancer influence. If sufficiently decremented, the growth rate dN(t)/dt may become negative, meaning that the tumor would regress. Equation [1], to be made appropriate for treated tumors, must therefore be changed to

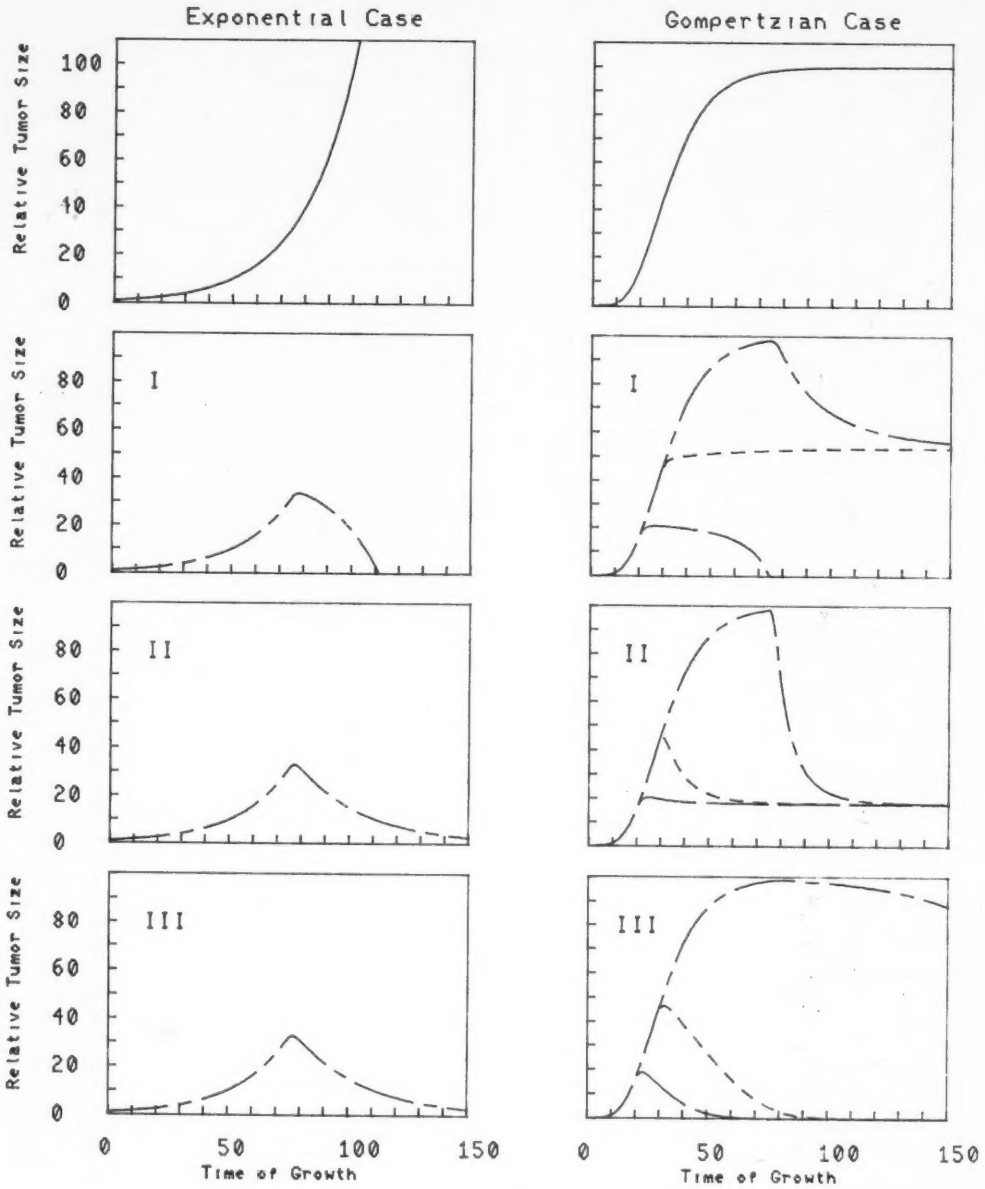
$$dN(t)/dt = GF(N)\cdot N(t) - D(N,t). \qquad [4]$$

The function D(N,t) represents the "growth-inhibitory influence" due to therapy, and must reflect both the level of the therapy at time t and the "sensitivity" to therapy of a tumor of size N. The function D(N,t) is not just the volume of cells killed by therapy, but reflects in addition the processes of dead cell removal, changes in vascularity, changes in benign cell content, and other volume alterations induced by treatment (20). Intuitively, equation [4] indicates that during the increment of time dt the tumor volume N(t) is incremented by the volume $GF(N)\cdot N(t)$, which is appropriate for an untreated tumor of size N(t), but is decremented by a certain volume D(N,t), which depends on the level of therapy. In other words, if the volume $GF(N)\cdot N(t)$ can be thought of as doubling in the time instant dt, the volume D(N,t) can be thought of as being instantaneously removed from the tumor during that same time interval. By the common clinical usage of the term "sensitivity," if a tumor N_1 is more sensitive to therapy than another tumor (or the same tumor at a different size) N_2, one would expect N_1 to regress faster than N_2 when exposed to a certain level of a given treatment. That is, the difference $GF(N)\cdot N(t) - D(N,t)$ would be more negative for N_1 than for N_2. This definition should be distinguished from that of the term "curability," for a tumor may be less "sensitive" than another in the sense of rate of regression, but more "curable" in terms of its inability to regrow following treatment. The difference between sensitivity and curability will be illustrated below.

By examining the implications of some simple alternative expressions for D(N,t), we may select the one most consistent with clinical and laboratory experience. Let us represent the level of therapy at time t by L(t). The term "level" does not refer strictly to "dose," for depending on the type of therapy used and such factors as route of administration or concurrent medication, L(t) may be related to the dose administered in a complicated fashion. For most types of therapy it is reasonable to assume that a higher dose level would be at least as growth-inhibitory as a lower dose level of the same agent. For the illustrations that follow (fig 3), let us examine the situation in which a constant dose level of effective therapy is frequently administered, so that L(t) reaches a plateau soon after the initiation of treatment.

The first form of D(N,t) to consider would result if the growth-rate inhibiting effect of a treatment is independent of the size of the tumor to which it is applied ($\partial D/\partial N = 0$). One mechanism which could lead to this result, for example, is if a fixed number of tumor cells were killed by a certain dose of therapy, regardless of the number of tumor cells present. While this alternative has long been regarded by experimentalists as unrealistic (21), we should, for the sake of completeness, examine its implications. Intuitively, this alternative suggests that the volume D(N,t), by which N(t) is decremented during the interval dt, is proportional only to L(t). The growth rate following therapy may therefore be represented by

$$dN(t)/dt = GF(N)\cdot N(t) - K_3\cdot L(t), \qquad [5]$$


Exponential Case
Gompertzian Case
Relative Tumor Size
100
80
60
40
20
0
I
II
III
0
50
100
150
Time of Growth

where K_3 is a constant. The patterns of growth predicted by equation [5] for both the exponential and Gompertzian cases are presented in figure 3, I. In the exponential case, because $GF(N) \cdot N(t)$ is equal to $K_1 \cdot N(t)$ which decreases with decreasing tumor size, the relative influence of L(t) is greater for smaller tumors. Hence, any level of L(t) that is sufficient in magnitude to initiate tumor regression would quickly reduce N(t) to 0. This is clearly unrealistic, for it implies that most therapy causing tumor regression should result in cure. The equation is equally unrealistic for Gompertzian growth, but for a different reason. Since the maximum value of $GF(N) \cdot N(t)$ occurs at the inflection point, the relative influence of L(t) would be least on tumors of intermediate size. According to equation [5], tumors of both small and large size, when $GF(N) \cdot N(t)$ is minimal, should be most sensitive to therapy. For tumors below their inflection point, any therapy capable of causing volume regression would necessarily lead to a complete eradication of disease. For large tumors, above the inflection point, the effect of therapy in causing tumor regression would increase with increasing tumor size. Hence, if equation [5] were correct, larger tumors should regress faster than those of intermediate size when exposed to equivalent L(t). Because these implications are inconsistent with clinical experience, equation [5] is seen to be unrealistic for both Gompertzian and exponential malignancies.

The currently popular alternative to the above "fixed-number kill" hypothesis is based on the belief that the growth-rate inhibiting effect of a treatment is proportional to the size of the tumor. This concept is founded primarily on detailed observations of exponentially growing tumor cell populations in which it has been determined that a fixed percentage of the tumor mass is killed by a given L(t), regardless of the size of the tumor at the time of treatment. On the basis of these observations, Skipper and co-workers (21) have long advocated this "percentage kill" (or "log kill") over the fixed-number kill hypothesis. Intuitively, this alternative would imply that the volume D(N,t), by which N(t) is decremented during dt, is a fraction of N(t), that fraction being proportional to L(t). The growth rate representing this alternative may be written

$$dN(t)/dt = GF(N) \cdot N(t) - K_4 \cdot L(t) \cdot N(t). \quad [6]$$

For exponential growth, where $GF(N) = K_2$, equation [6] may be rewritten

$$dN(t)/dt = \{K_2 - K_4 \cdot L(t)\} \cdot N(t). \quad [6a]$$

This equation describes a reasonable pattern of exponential regression (fig 3, II) in which "cure" [defined as the reduction of N(t) below the volume of a single cell] is assured only if treatment is continued for a sufficiently long period of time with $K_4 \cdot L(t) > K_2$. Indeed, laboratory experience with L1210 and other "exponentially" growing tumors has confirmed this pattern of regression. For human tumors which obey Gompertzian growth kinetics, however, equation [6] is less satisfactory, for $GF(N) \cdot N(t)$ becomes smaller as N(t) grows above the inflection point, while $L(t) \cdot N(t)$ would simultaneously increase in magnitude. Hence, according to the log-kill hypothesis, very large tumors would be inordinately sensitive to therapy in terms of rate of regression (fig 3, II). It is not generally realized that for tumors growing in a Gompertzian manner, the log-kill hypothesis implies that the largest tumors should be the most sensitive to therapy both in terms of number of cells killed and rate of regression. From a clinical standpoint this is clearly unrealistic. Therefore, while we may agree with others that equation [6] is appropriate for exponentially growing tumors, it is clear that the log-kill concept as expressed in equation [6] is not generally applicable to most clinical neoplasms.

In choosing an alternative to this equation we must take care to find a concept that, in addition to fitting the observed exponential regression of exponentially growing tumors, fits clinical experience as well. An alternative that meets these requirements is that the growth-inhibiting effect of a treatment is proportional to the growth rate of an untreated

FIGURE 3.—Hypothetic growth patterns generated by 3 alternative forms of D(N,t), the growth-inhibition due to therapy. On the left side are examples for the exponential case with unperturbed growth illustrated for comparison at the top; on the right side are examples for the Gompertzian case. For the Gompertzian case, different line-types refer to different times of initiation of therapy: before the inflection point (– – –), at around the inflection point (- - -), and after the inflection point (– - – -). In all cases L(t) is 0 before initiation of therapy, and a constant positive No. thereafter. I = patterns resulting from use of equation [5]. In the exponential case, any therapy capable of causing tumor regression should necessarily result in cure; in the Gompertzian case, the tumor would exhibit its minimum sensitivity at the inflection point, with increasing sensitivity at larger sizes. II = patterns resulting from use of equation [6]. In the exponential case, the regression curve would be exponential; in the Gompertzian case, the sensitivity to therapy would increase with increasing tumor size. III = patterns resulting from use of equation [7]. In the exponential case, the regression curve is identical to that generated by equation [6]; in the Gompertzian case, the maximum sensitivity to therapy is found at the inflection point, with less sensitivity at smaller sizes and least sensitivity at large tumor sizes.

tumor of size N(t). This means that the volume removed is proportional to the GF times the total tumor volume, which may be written

$$dN(t)/dt = GF(N)\cdot N(t) - K_s\cdot L(t)\cdot GF(N)\cdot N(t). \quad [7]$$

In the exponential case, equation [7] reduces to equation [6a] which, as we have already seen, is a reasonable model for exponential tumor regression. In other words, equation [7] is equivalent to the log-kill hypothesis when applied to exponentially growing tumors, and is therefore entirely consistent with the laboratory results cited above. In the Gompertzian case, $GF(N)\cdot N(t)$ is maximum for intermediate-sized tumors, so that the influence of therapy, given by $K_s\cdot L(t)\cdot GF(N)\cdot N(t)$, would decrease as the tumor grows above the middle-size range toward larger sizes. Hence, unlike the log-kill hypothesis, equation [7] is consistent with the observation that large human tumors are less responsive to therapy than tumors of intermediate size.

In addition to being consistent with clinical experience, equation [7] has proven applicable to experimental tumors as well. Figure 4 shows examples of the application of equation [7] to tumor-size growth curves actually observed in the laboratory. In all cases the predicted curves are remarkably similar to those drawn from observed measurements. Ap-

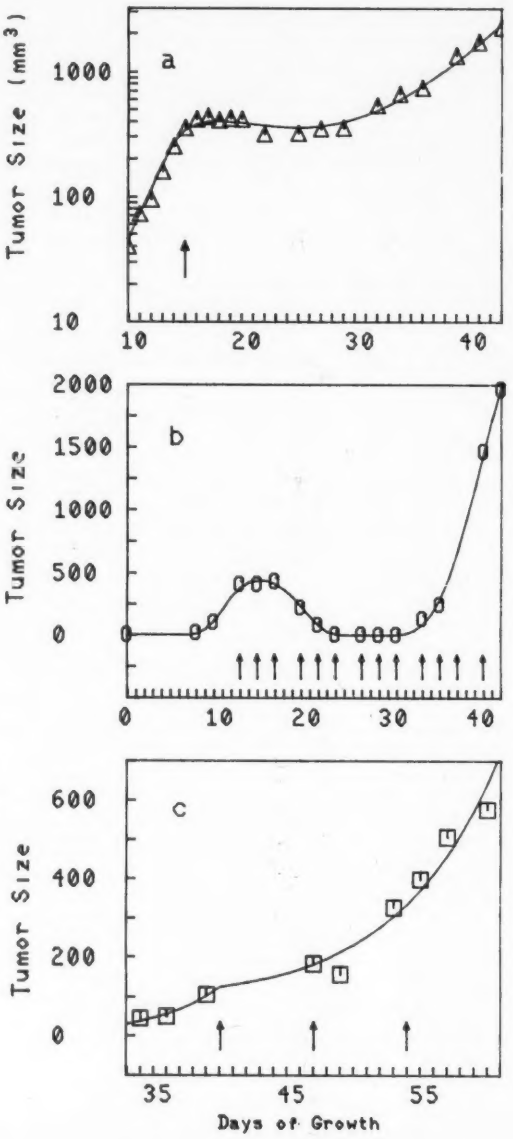


FIGURE 4.—Examples of experimental data and corresponding curves generated by equation [7]: *a*. △ = average volumes for a group of rat hepatomas (ref 22) treated at the time marked by the arrow with 1500 rads of radiotherapy; solid line = curve generated by equation [7] which is seen to accurately fit the data points (ref 20). Equations used are:

$$dN(t)/dt = \{1-Y(t)\}\cdot B\cdot \log\{\exp(11.91)/N(t)\}\cdot N(t)$$
$$B = 0.05427$$
$$Y(t) = 0 \text{ if } t<14, \text{ otherwise } P\cdot(t-14)^{\circ}\cdot\exp\{(14-t)/F\}$$
$$P = 0.6222$$
$$E = 0.5215$$
$$F = 13.61$$

b. ○ = volume measurements for a single rat Adenocarcinoma 13762 treated with melphalan at the times indicated by the arrows. Regrowth with therapy is presumably due to a cell line with different biochemical sensitivity to the drug. Solid line = curve generated by simultaneous use of 2 equations of the form of equation [7], 1 equation representing the more sensitive cell line and the other (with different K_s) the less sensitive cell line. Generated curve is again seen to accurately fit the observed data. Equations used are:

$$N(t) = N_1(t)+N_2(t)$$
$$\{N_2(0)/N_1(0)\} = 0.000268$$
$$dN_1(t)/dt = \{1-Y_1(t)\}\cdot B\cdot\log\{\exp(9.76)/N(t)\}\cdot N_1(t)$$
$$dN_2(t)/dt = \{1-Y_2(t)\}\cdot B\cdot\log\{\exp(9.76)/N(t)\}\cdot N_2(t)$$
$$B = 0.1072$$
$$Y_1(t) = 0 \text{ if } t<13, \text{ otherwise } (1.545)\cdot\tanh\{(t-13)/1.933\}$$
$$Y_2(t) = 0 \text{ if } t<13, \text{ otherwise } (0.3245)\cdot\tanh\{(t-13)/1.933\}$$

c. □ = measured volumes for a single mouse breast cancer treated with cyclophosphamide at the times indicated by the arrows; solid line = curve generated by equation [7] which is seen to accurately fit the data. Equations used are:

$$dN(t)/dt = \{1-Y(t)\}\cdot B\cdot\log\{\exp(19.93)/N(t)\}\cdot N(t)$$
$$B = 0.0130$$
$$Y(t) = 0 \text{ if } t < 40, \text{ otherwise } 0.5554$$

plying equation [7] to published data on the response of a rat hepatoma to radiotherapy (22) yields a close approximation to the shape of the actual data points (20). Similarly, the patterns of response of two different experimental mammary adenocarcinomas to chemotherapy[3,4] are also well-matched by equation [7].

The most convincing demonstration of the preferability of equation [7] to the log-kill hypothesis is found in the examination of the growth pattern of tumors exposed to L(t) sufficient to cause growth inhibition but insufficient to cause regression (fig 5). According to the log-kill hypothesis as applied to Gompertzian growth, when the value $GF(N) \cdot N(t)$ is equivalent to the "kill" volume $K_4 \cdot L(t) \cdot N(t)$, the tumor should exhibit no further growth. Therefore, if a tumor is treated with an L(t) insufficient to cause regression, it should grow only until it reaches a plateau size, when $K_2 \cdot \ln\{N(\infty)/N(t)\}$ equals the constant $K_4 \cdot L(t)$. This new plateau should be smaller than the hypothetic ideal size for that tumor if left untreated. On the other hand, according to equation [7], as $GF(N) \cdot N(t)$ grows smaller with increasing size, so does $K_5 \cdot L(t) \cdot GF(N) \cdot N(t)$. Thus, the treated tumor should approach its ideal maximum size, but more slowly than in the untreated state. Observation of tumors exposed to low, continuous doses of therapy indeed confirms that equation [7] better predicts the growth curves of such tumors than the log-kill hypothesis.[3]

On the basis of experimental results, therefore, we contend that the concept contained in equation [7] describes a relationship between tumor size and sensitivity to therapy that is more consistent with clinical and laboratory data than the conventional concept. The importance of this approach is that the application of our equations may have profound clinical implications. These implications are especially apparent when we recognize that for tumors small enough to be below their inflection point, $GF(N) \cdot N(t)$ decreases in magnitude with decreasing tumor size (figs 1 and 2). Thus, by equation [7], the sensitivity to therapy in terms of rate of regression for a given L(t) would decrease with decreasing tumor size, even though the GF is increasing. It is important to recognize that even if we were to accept the log-kill equation [6] for Gompertzian tumors, this relationship would still hold, because $\{GF(N) - K_4 \cdot L(t)\} \cdot N(t)$ would be less negative for a smaller N(t). That is, for a given L(t), the rate of regression of a small tumor (below its inflection point) will be less than that of a tumor of intermediate size.

This does not necessarily mean that the smaller tumor is less "curable" than a larger one, for although its rate of regression may be smaller, it is closer to a limiting volume beyond which it may not be capable of regrowth (in some cases this may be the volume of a single cell). With very effective therapy, as is seen in many experimental settings, a small tumor may be cured by a dose of therapy which would not cure a larger tumor of equivalent histology. However, these concepts do imply that a dose schedule capable of causing a dramatic rate of regression of a tumor of intermediate size may not be sufficient to cure a small tumor when applied over a duration of therapy limited in time by considerations of host tolerance.

This important clinical implication is illustrated in figure 6, in which six courses of therapy with equal L(t) were used in solving equation [7] to simulate the response of a hypothetic tumor to a MOPP-type regimen (13).[5] As is easily seen, the course of therapy administered to the small tumor volume caused a shallow rate of regression, so that the tumor was not eradicated, but was capable of regrowth when therapy was discontinued. Several additional courses of therapy at the same L(t) would also cause shallow rates of regression and therefore would be ineffective in preventing recurrence. This is, of course, consistent with such observations as the failure of additional cycles of chemotherapy to improve prognosis once CR is induced in Burkitt's lymphoma (23). Maintenance therapy at a lower L(t) than the six induction cycles would be even less effective. This would explain the failure of maintenance chemotherapy to decrease the rate of recurrence of cases of Hodgkin's disease in which a documented CR has been achieved (24).

In the case of adjuvant chemotherapy, equation [7] would suggest the use of more intense schedules, higher doses, and perhaps more prolonged therapy than might otherwise be employed. While prolonged, low-dose regimens may be effective in lengthening the disease-free interval prior to recurrence, the shallow rate of regression may not be sufficient to eradicate all cells, so that the eventual rate of recurrence would be unaffected or only slightly decreased.

The most efficient therapy for advanced tumors would make maximum use of equation [7], applying moderately intense therapy initially, when the tu-

[3]Experimental data kindly supplied by Dr. A. E. Bogden, Mason Research Institute, Worcester, Mass.

[4]Experimental data kindly supplied by Dr. D. S. Martin, Department of Surgery, Catholic Medical Center, Woodhaven, NY.

[5]MOPP = mechlorethamine, vincristine, prednisone, and procarbazine.

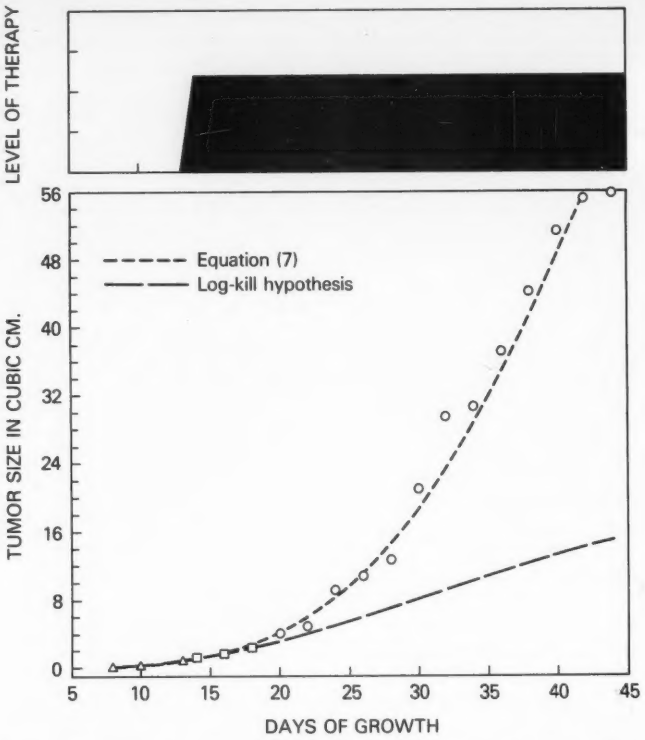


FIGURE 5.—Comparative curves generated by equations [6] (log-kill hypothesis) and [7] with corresponding experimental data. Unperturbed Gompertzian curve (equation [3]) was fit to 3 measurements (△) of an individual Adenocarcinoma 13762 prior to institution on Day 13 of continuous 5-FU therapy. This curve fit was accomplished by a previously published method (ref 16) that yields the estimates $K_2 = 0.04334$ and $N(\infty) = \exp(13.15)$. Both equations [6] and [7] were then fit to 3 data points observed after initiation of therapy (□), yielding estimates of $K_4 \cdot L(t) = 0.09345$ and $K_5 \cdot L(t) = 0.2245$, respectively. Since, even in Gompertzian growth, GF does not change appreciably over the small range of N(t) represented by the 3 points (□), both equations [6] and [7] fit the curve equally well over this interval. However, when the equations are solved for subsequent points, in an effort to predict the future growth of the tumor, the resulting lines differ dramatically (*see* text). It is emphasized that these lines were drawn by computer prior to measurement of subsequent points. Subsequent measurements were then obtained and plotted with the symbol ○; they are seen to match the line generated by equation [7] with remarkable accuracy. Many similar experiments have confirmed the preferability of equation [7] to the log-kill hypothesis.

mor may be above its inflection point, but intensifying therapy after the attainment of CR, when the tumor is probably below its inflection point. This is, of course, similar to the "late-intensification" approach recently proposed for the therapy of acute myelogenous leukemia (25). The possible value of this approach is illustrated by a simulation of two plans of therapy in causing regression of a tumor which is above its inflection point at the initiation of treatment (fig 7). The treatment plan illustrated in figure 7A leads to steady tumor regression and eradication of disease. Using the same total amount of therapy [that is, the same total area under the graph of L(t)], the plan illustrated in figure 7B fails to reduce the tumor volume below the size of a single cell. Therefore, the tumor treated by plan B, which is the dose schedule most often employed in the clinic, is seen to regrow after the termination of therapy.

It is emphasized that this regrowth is due to insufficient therapy at a time of "kinetic" therapy resistance, and not resistance at a biochemical, intracellular level. Hence, as is seen in some cases of leukemia and malignant lymphoma, the recurrent tumor may be responsive to the reapplication of the original treatment. It is not denied here that biochemical resistance can and often does occur. There are cases, however, in which tumor recurrence may be due not to biochemical resistance but to the suboptimal use of the original agents. This

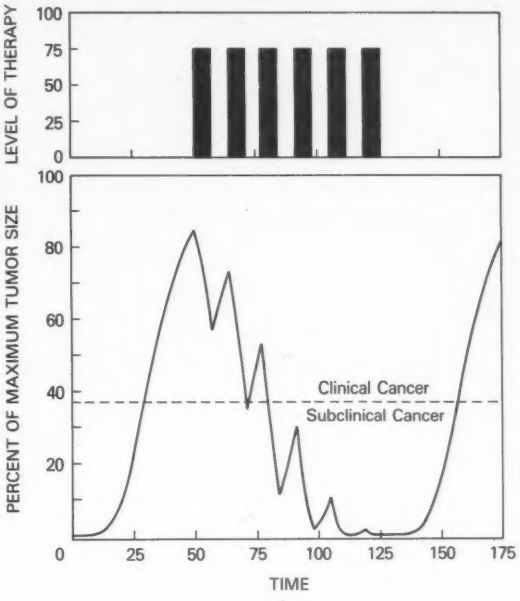


FIGURE 6.—Equation [7] solved using values for L(t) presented in the upper graph, simulating the effect of a MOPP-type regimen on a hypothetic tumor. The first 4 cycles of therapy are seen to cause rapid tumor regression, but the 5th and 6th cycles, administered at a time of relatively slow growth rate, are less effective, so that the tumor is not eradicated but is capable of regrowth when therapy is discontinued.

important conclusion is not limited to the application of equation [7], for it is based on the small tumor's decreased rate of regression for a given L(t) that is described both by equation [7] and the log-kill hypothesis as expressed in equation [6].

The treatment plan A is appealing not only because of its success in the above simulation but on purely clinical grounds as well. If therapy is initiated at a high level of intensity with tapering as treatment is continued beyond the attainment of CR, the ability to follow tumor volume regression is lost so that the L(t) chosen during CR may be insufficient to maintain the pattern of response. If, however, in consideration of host tolerance, it is possible to intensify therapy during CR, one may be reasonably assured that the pattern of tumor regression will be maintained, except for the emergence of biochemically resistant cells. It should be emphasized that plan A would be useful in the clinic even if the tumor under treatment did not obey strictly Gompertzian kinetics, because the relationship between tumor size and sensitivity to treatment described in this paper is valid for any sigmoid ("S"-shaped) growth curve. In fact, even if we were to accept the log-kill principle for tumors following sigmoid growth curves, we would still find that plan A would be a reasonable approach to treatment design.

It is important to note that according to our concept, after the attainment of CR, high-dose therapy of a relatively brief duration may be preferable to prolonged low-dose therapy in decreasing rates of recurrence. Microscopic disease residual during CR may require but a brief period of rapid regression to be eradicated, whereas a prolonged period of less rapid regression may be insufficient. That is, while the microscopic tumor may be relatively "insensitive" in terms of rate of regression, it may be quite "curable" in terms of being near the volume beyond which it may be incapable of further regrowth. In this regard, if several agents are to be combined in an effort to avoid biochemical drug resistance, they should be administered concurrently only if there is reason to anticipate some synergism or if dose modifications would not be required. If dose modifications are required to use agents concurrently in the treatment of microscopic disease, it would probably be preferable to administer them in rapid alternating sequences so as to be able to give the maximum L(t) for each agent.

Our hypothesis is offered not as the only reasonable alternative to the conventional view, but as one simple, experimentally testable, and clinically applicable alternative that has the added advantage of being consistent with a large body of clinical and laboratory experience. Numerous modifications of equation [7] may be dictated by further experience in the application of our methods to clinical practice. For example, the co-existence of several cell lines with different sensitivities to therapy, including complete biochemical resistance, may be found in some cases. This may require the simultaneous use of two or more equations of the form of equation [7], as is illustrated in figure 4b. Applying the equation to tissues that retain considerable sensitivity near their $N(\infty)$ would require additional alterations. Such modifications, however, do not alter the underlying concept that small tumors may have decreased sensitivity to treatment quite independent of biochemical resistance at the intracellular level. Since the clinical implications of the relationship between tumor size and sensitivity to therapy may have considerable impact on the design of anticancer regimens, we propose that a careful reevaluation of the currently popular "small-is-sensitive" concept is clearly in order.

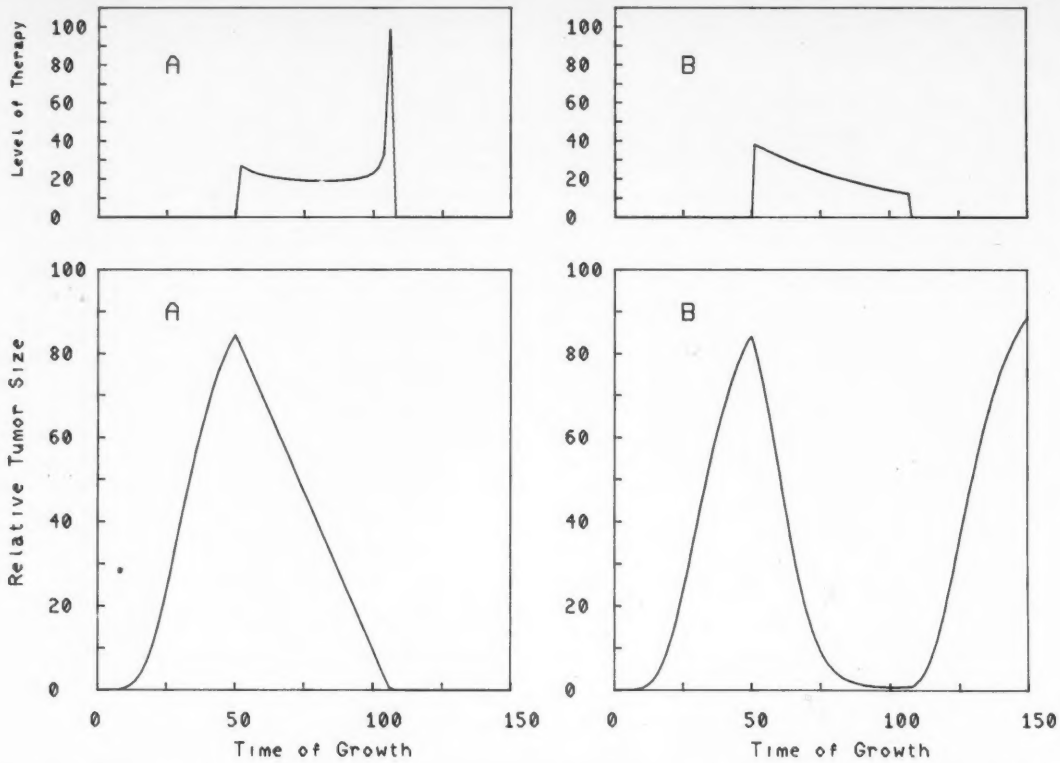


FIGURE 7.—Simulation of the effect of 2 plans of therapy using equation [7] in causing regression of an advanced tumor. Plan A applies a therapy level just sufficient to maintain a steady volume regression, saving a "spike" of intense therapy for late in the course when the small tumor is relatively therapy-resistant; this plan reduces tumor volume below the volume of a single cell, resulting in "cure." Plan B uses the same total amount of therapy but gives the most intense treatment initially, with tapering of dose levels as CR is induced. When the tumor is small and relatively therapy-resistant, therapy administered is insufficient to reduce the volume below that of a single cell. Therefore, the tumor treated by plan B regrows after the termination of treatment.

REFERENCES

1. SCHABEL FM, JR. Concepts for systemic treatment of micrometastases. Cancer 35:15–24, 1975.
2. FREI E III. A Commentary: Selected considerations regarding chemotherapy as adjuvant in cancer treatment. Cancer Chemother Rep 50:1–5, 1966.
3. MAUER AM, and SIMONE JV. The current status of the treatment of childhood acute lymphoblastic leukemia. Cancer Treat Rev 3:17–41, 1976.
4. SIEBER SM. Commentary: Cancer chemotherapeutic agents and carcinogenesis. Cancer Chemother Rep 59:915–918, 1975.
5. SCHEIN PS, and WINOKUR SH. Immunosuppressive and cytotoxic chemotherapy: long-term complications. Ann Intern Med 82:84–95, 1975.
6. CANELLOS GP, DEVITA VT, JR, ARSENEAU JC, ET AL. Carcinogenesis by cancer chemotherapeutic agents: second malignancies complicating Hodgkin's disease in remission. Recent Results Cancer Res 49:108–114, 1974.
7. FISHER B, CARBONE P, ECONOMOU SG, ET AL. L-Phenylalanine mustard in the management of breast cancer: a report of early findings. N Engl J Med 292:117–122, 1975.
8. BONADONNA G, BRUSAMOLINO E, VALAGUSSA P, ET AL. Combination chemotherapy as an adjuvant treatment in operable breast cancer. N Engl J Med 294:405–410, 1976.
9. TORMEY DC. Combined chemotherapy and surgery in breast cancer: a review. Cancer 36:881–892, 1975.
10. HIGGINS GA, JR, HUMPHREY E, JULER GL, ET AL. Adjuvant chemotherapy in the surgical treatment of large bowel cancer. Cancer 38:1461–1467, 1976.
11. LUCE JK, THURMAN WG, ISAACS BL, ET AL. Clinical trials with the antitumor agent 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (NSC-45388). Cancer Chemother Rep 54:119–124, 1970.
12. PATERSON AHG, and MCPHERSON TA. Malignant melanoma: a pilot study of adjuvant chemo-immunotherapy. *In* The Adjuvant Therapy of Cancer. Amsterdam, Elsevier/North-Holland Publishing Co. In press.
13. DEVITA VT, JR. Combined drug treatment of Hodgkin's disease: remission induction, remission duration, and survival; an appraisal. Natl Cancer Inst Monogr 36:373–379, 1973.
14. NKRUMAH FK, and PERKINS IV. Relapse in Burkitt's lymphoma. Int J Cancer 17:455–460, 1976.

15. LEVENTHAL BG, LEVINE AS, GRAW RG, JR, ET AL. Long-term second remissions in acute lymphatic leukemia. Cancer 35:1136–1140, 1975.
16. NORTON L, SIMON R, BRERETON HD, ET AL. Predicting the course of Gompertzian growth. Nature 264:542–545, 1976.
17. STEEL G. Cytokinetics of neoplasia. *In* Cancer Medicine (Holland JF, and Frei E III, eds). Philadelphia, Lea and Febiger, 1973, pp 125–140.
18. LAIRD AK. Dynamics of growth in tumors and normal organisms. Natl Cancer Inst Monogr 30:15–28, 1969.
19. GOMPERTZ B. On the nature of the function expressive of the law of human mortality, and on the new mode of determining the value of life contingencies. Philos Trans R Soc Lond [Biol] 115:513–585, 1825.
20. NORTON L, and SIMON R. Growth curve of an experimental solid tumor following radiotherapy. J Natl Cancer Inst 58:1735–1741, 1977.
21. SKIPPER HE, SCHABEL FM, JR, and WILCOX WS. Experimental evaluation of potential anticancer agents. XIII. On the criteria and kinetics associated with "curability" of experimental leukemia. Cancer Chemother Rep 35:1–111, 1964.
22. LOONEY WB, TREFIL JS, SCHAFFNER JC, ET AL. Solid tumor models for the assessment of different treatment modalities. I. Radiation-induced changes in growth rate characteristics of a solid tumor model. Proc Natl Acad Sci USA 72:2662–2666, 1975.
23. ZIEGLER JL. Chemotherapy of Burkitt's lymphoma. Cancer 30:1534–1540, 1972.
24. YOUNG RC, CANELLOS GP, CHABNER BA, ET AL. Maintenance chemotherapy for advanced Hodgkin's disease in remission. Lancet 1:1339–1343, 1973.
25. BODEY GP, FREIREICH EJ, GEHAN E, ET AL. Late intensification therapy for acute leukemia in remission. JAMA 235:1021–1025, 1976.

Differential Distribution of Antitumor Agents in Primary and Secondary Tumors [1,2]

M. G. Donelli,* T. Colombo, M. Broggini, and S. Garattini [3,4]

SUMMARY

The differential distribution of a series of antineoplastic agents in metastatic tissues compared to their respective primary tumors has been investigated in one rat and two mouse experimental tumor systems, ie, the intramuscular Lewis lung carcinoma (3LL) of C57BL/6 mice, which gives rise to spontaneous lung metastases, the intratibial Sarcoma 180 (S180) of CD_1 mice, which induces macroscopic metastases to the lymph nodes, and the Walker 256 carcinosarcoma of CD rats, which also metastasizes to the lymph nodes. The results described in this paper show that the concentrations of adriamycin, daunorubicin, cyclophosphamide and its alkylating metabolites, hydroxyurea, 1-methyl-1-nitrosourea, and 6-mercaptopurine are much higher in the pulmonary metastases of 3LL and/or in the lymph node metastases of S180 than the concentrations measured in the primary tumor. In the Walker 256 tumor system the distribution of adriamycin appears to follow the same pattern observed for the mouse tumors. Only for methotrexate (in the 3LL tumor) is the difference in the concentrations at the two sites not so evident. These findings are discussed in relation to the comparatively greater sensitivity of metastases to chemotherapy.

[Cancer Treat Rep 61:1319–1324, 1977]

In experimental and clinical studies of malignant diseases, metastases have been observed to respond to chemotherapy better than the primary tumor from which they are derived (1–6). The reasons for this differential sensitivity may be ascribed to variations in biochemical, physiologic, and environmental conditions of primary and secondary neoplasms, among which particular attention has been focused in experimental studies on the proliferative state of the tumor cell population (7,8). In fact, it is generally accepted that the growth curve of cancer cells follows a Gömpertzian function (9–11) which means that the mass doubling time becomes progressively longer as the neoplasm increases in size; the smaller the neoplastic mass, the greater its doubling capacity. Because a direct correlation was observed between tumor cell doubling time and sensitivity to drugs inhibiting cell multiplication (3,12–14), metastases which are generally smaller than the respective primary tumors, and therefore in a more rapid proliferative state, should be more sensitive to chemotherapeutic agents. These observations are consistent with the conclusion that the proliferative state of a tumor cell population is a determinant of drug sensitivity and may explain, at least partially, the different response of primary and secondary outgrowths.

Another factor which could account for the greater drug efficacy on metastases than on primary tumor growth may be represented by the drug concentrations attained in the primary tumor

[1]Received Dec 27, 1976; revised May 20, 1977; accepted May 25, 1977.

[2]Supported by contract N01-CM-23242 (Division of Cancer Treatment [DCT]) and Public Health Service grant CA-12764 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3]Istituto di Ricerche Farmacologiche "Mario Negri," Milan, Italy.

[4]The skillful technical assistance of Mariangela Mainini and the help of Brunella Barbieri are gratefully acknowledged. We thank Farmitalia and Simes (Milan, Italy), Asta-Werke (Bielefeld, W. Germany), and the Developmental Therapeutics Program, DCT, NCI (Bethesda, Md) for providing the drugs used in this study.

**Reprint requests to:* M. G. Donelli, MD, Istituto di Ricerche Farmacologiche "Mario Negri," Via Eritrea 62, 20157 Milan, Italy.

and metastases; preliminary observations by this laboratory have indicated that antitumor drugs preferentially accumulate in the metastases of the mouse (15).

To our knowledge little is reported in the literature about drug distribution in tumors in different sites and particularly in metastatic tissues compared to their respective primary tumors. Therefore, it was considered of interest to investigate the disposition of a series of chemotherapeutic agents in primary and secondary outgrowths of two transplantable tumor systems in the mouse and one in the rat; the mouse intramuscular Lewis lung carcinoma (3LL), which gives rise to spontaneous lung metastases (16), the mouse intratibial Sarcoma 180 (S180), which induces macroscopic metastases to the lymph nodes (17), and the rat Walker 256 carcinosarcoma, which also metastasizes to the lymph nodes.

MATERIALS AND METHODS

C57BL/6 male and CD_1 female mice (22 ± 2 g) and CD male rats (150 ± 10 g), obtained from Charles River Breeding Laboratories (Italy), were used for these experiments. C57BL/6 mice received an im transplant of 2×10^5 viable cells of the syngeneic 3LL, which was maintained by im passages in the same strain every 2 weeks. The mice were used 25 days after tumor transplant, when the primary tumor reached approximately its maximum growth (7.2 ± 1.1 g) and its pulmonary metastases numbered about 27 ± 2.7 per mouse and had an average weight of 169 ± 35 mg per mouse.

CD_1 mice received an intratibial transplant of 5×10^5 viable cells of S180, maintained in the ascites form by ip serial passages in the same strain every week. The mice were used 55 days after tumor transplant, when the primary tumor reached 7.5 ± 0.3 g and its lymph node metastases weighed approximately 195 ± 30 mg for the renal and 370 ± 42 mg for the iliac metastases. This tumor system has been described by Franchi et al (17).

CD rats received an im transplant of 10^6 viable cells of Walker 256 carcinosarcoma maintained in solid form by sc serial passages in the same strain every 2 weeks. The rats were used 15 days after tumor transplant, when the primary tumor reached 31 ± 7 g and its lymph node metastases (renal, iliac, and periaortic) weighed 4.0 ± 1.2 g.

The following drugs were used: adriamycin, daunorubicin, methotrexate, cyclophosphamide, hydroxyurea, 1-methyl-1-nitrosourea, and 6-mercaptopurine.

After iv injection, the drug concentrations in the primary tumors and metastases were assayed at 1, 5, 15, 30, 60, 180, and 480 minutes and for some drugs also at 24, 48, and 72 hours after injection (five animals per point) according to different biochemical methods. A fluorimetric procedure for adriamycin and daunorubicin as described by Schwartz (18) was used with minor modifications. Recovery was 75% ± 3% and sensitivity was about 1 μg/g tissue.

A fluorimetric procedure for methotrexate described by Chakrabarti and Bernstein (19) was used with minor modifications for adaptation to tissues. Recovery was 90% ± 4% and sensitivity was about 1 μg/g tissue.

A gas chromatographic procedure recently set up in this Institute by Facchinetti et al (20) was used for cyclophosphamide and a colorimetric procedure for its alkylating metabolites, as described by Friedman and Boger (21) with minor modifications. Recovery was 88% ± 4% and 91% ± 3% respectively and sensitivity was about 0.1 μg/g and 10 nmols nitrogen mustard equivalent/g tissue.

A colorimetric procedure was used for hydroxyurea as described by Bolton et al (22) with minor modifications for adaptation to tissues. Recovery was 90% ± 7% and sensitivity was about 1 μg/g tissue.

A colorimetric procedure was used for 1-methyl-1-nitrosourea as described by Forist (23) for nitrosourea compounds with minor modifications. Recovery was 90% ± 4% and sensitivity was about 1 μg/g tissue.

A spectrophotometric procedure was used for 6-mercaptopurine as described by Donelli et al (24). Recovery was 60% ± 9% and sensitivity was about 3 μg/g tissue. The drug concentrations in the primary tumor were generally measured on the whole tissue, without discarding the necrotic part. For adriamycin, however, a separate measurement on the viable and necrotic part was performed in order to assess the importance of the presence of necrosis in the estimate of drug concentrations in the tumor.

The area under the concentration versus time curve (AUC) was measured by trapezoidal integration up to the last time at which the drug was detectable. The significance of the difference between the levels found in primary tumors and metastases was assessed by the Student's t test or by an analysis of variance on the half-life and AUC values. The half-life was assessed by calculating the significance of the regression using Fisher's F ratio (according to the least-squares method). For the assessment of adriamycin antitumor activity the weight inhibition of primary tumor and metastases together with their number and takes were recorded. The significance of the difference between

controls and treated animals was assessed by the Student's t test or by the Fisher's exact test.

RESULTS

The results presented in figure 1 indicate that the concentrations of adriamycin in pulmonary metastases of the im 3LL after iv injection of graded doses of this antitumor antibiotic are always much higher (at least double) than the concentrations measured in the primary tumor, either expressed per g of total tumor tissue or per g of viable tissue. Similar results, summarized in table 1, are obtained for the other compounds investigated (daunorubicin, hydroxyurea, 1-methyl-1-nitrosourea, and cyclophosphamide).

For cyclophosphamide the results are particularly remarkable: 1 minute after injection, about seven times the amount of unchanged compound is found in the metastatic nodules; whereas no drug metabolites are detectable in the primary tumor, > 100 nmols nitrogen mustard equivalents are present in the metastases. The methotrexate concentrations (table 1), as expressed by the AUC values, do not reveal significant differences between the two sites, although methotrexate reaches a much higher peak in the pulmonary metastases than in the primary tumor.

Approximately the same results are obtained with the intratibial S180, the other metastasizing tumor investigated. Drug concentrations in the macroscopic lymph node metastases are consistently higher than the primary tumor levels (table 2). In fact, adriamycin, which is not detectable in the primary tumor, reaches measurable concentrations in both the renal and iliac metastatic lymph nodes. For 6-mercaptopurine the difference between primary tumor and metastases is particularly evident considering the renal metastases; even methotrexate, whose distribution pattern in the 3LL and its pulmonary metastases showed no marked differences, concentrates in the metastatic lymph nodes, at least at the renal site, much more than in the primary intratibial S180.

In Walker 256 carcinosarcoma, an experimental tumor system of the rat (fig 2), the distribution of adriamycin appears to follow the same pattern observed for the mouse tumors; in fact, the drug levels detectable in metastases are about double those in the primary im tumor.

Results of the antitumor and antimetastatic activity of adriamycin in the 3LL and Walker 256 carcinosarcoma models are presented in table 3; it is evident that a much greater inhibition of growth occurs for secondary compared to primary neoplasms and even a complete disappearance of metastatic outgrowths is observed for the Walker 256 tumor.

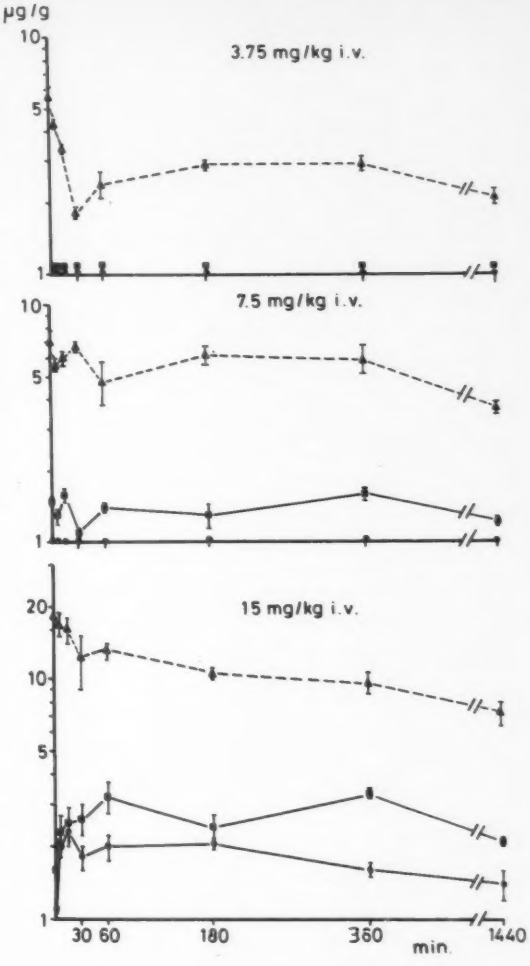


FIGURE 1.—Adriamycin levels 25 days after tumor transplantation in im 3LL and its pulmonary metastases after iv injection of 3.75, 7.5, and 15 mg/kg in C57BL/6 mice. ●——● = primary tumor (whole); ■——■ = primary tumor (viable part); ▲- - -▲ = metastases.

DISCUSSION

Our findings that chemotherapeutic agents generally distribute to metastatic outgrowths more than in the primary animal tumors are in good agreement with the report by Houghton et al (25) who found that cyclophosphamide uptake in pulmo-

TABLE 1.—Levels of adriamycin, daunorubicin, hydroxyurea, 1-methyl-1-nitrosourea, cyclophosphamide and its alkylating metabolites, and methotrexate in im 3LL and its pulmonary metastases

Drug* and kinetic parameters	Primary tumor	Lung metastases
Adriamycin		
3.75 mg/kg:		
Peak level (μg/g ± SE)	<1	5.6±0.6†
AUC (μg/g × mins) after 24 hrs	<1	2693±325†
7.5 mg/kg:		
Peak level (μg/g ± SE)	<1	7.0±0.8†
AUC (μg/g × mins) after 24 hrs	<1	7154±962†
15 mg/kg:		
Peak level (μg/g ± SE)	2.3±0.5	18.0±2.4†
AUC (μg/g × mins) after 24 hrs	2306±199	13,194±1285†
Daunorubicin (15 mg/kg)		
Peak level (μg/g ± SE)	2.9±0.5	30.2±2.1†
AUC (μg/g × mins) after 24 hrs	3515±597	19,286±2239†
Hydroxyurea (300 mg/kg)		
Peak level (μg/g ± SE)	95.2±3.8	233±12†
AUC (μg/g × mins) after 6 hrs	26,493±1925	64,237±2717†
1-Methyl-1-nitrosourea (20 mg/kg)		
Peak level (μg/g ± SE)	<1	12.2±0.6‡
AUC (μg/g × mins) after 30 mins	61±11	129±11‡
Cyclophosphamide (50 mg/kg)		
Peak level (μg/g ± SE)	9.7±4.0	66.4±9.7†
AUC (μg/g × mins) after 6 hrs	871±346	1629±182†
β $T^{1}/_{2}$ (mins)	114 (79–165)	112 (87–153)
Cyclophosphamide metabolites		
Peak level (nmols nitrogen mustard equivalent/g ± SE)	<10	154.3±11.9†
AUC (nmols nitrogen mustard equivalent/g × mins) after 6 hrs	<10	37,558±6092†
β $T^{1}/_{2}$ (mins)	—	158 (99–201)
Methotrexate (15 mg/kg)		
Peak level (μg/g ± SE)	5.5±1	11±0.6‡
AUC (μg/g × mins) after 24 hrs	3093±657	1900±209
β $T^{1}/_{2}$ (mins)	205 (167–245)	238 (178–282)

*Injected iv in C57BL/6 mice with im 3LL 25 days after tumor transplantation.
†$P < 0.01$.
‡$P < 0.05$.

TABLE 2.—Levels of adriamycin, methotrexate, and 6-mercaptopurine in intratibial S180 and its lymph node metastases

Drug* and kinetic parameters	Primary tumor	Pararenal metastases	Iliac metastases
Adriamycin (15 mg/kg)			
Peak level (μg/g ± SE)	<1	3.6±0.05†	2.6±0.2†
AUC (μg/g × mins) after 24 hrs	<1	2435±339†	3619±537†
Methotrexate (15 mg/kg)			
Peak level (μg/g ± SE)	4.1±0.1	5.9±1.2	6.0±1.3
AUC (μg/g × mins) after 1 hr	103±9	214±26‡	193±22‡
6-Mercaptopurine (100 mg/kg)			
Peak level (μg/g ± SE)	17±3	31.6±2.7‡	21.6±1.1
AUC (μg/g × mins) after 6 hrs	521±48	4421±578†	2338±370†

*Injected iv in CD_1 mice with intratibial S180 55 days after tumor transplantation.
†$P < 0.01$ (vs primary tumor).
‡$P < 0.05$ (vs primary tumor).

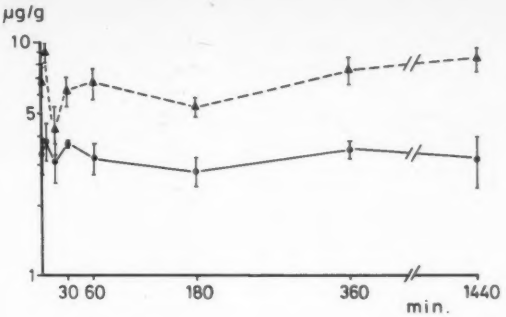


FIGURE 2.—Adriamycin levels 15 days after tumor transplantation in im Walker 256 carcinosarcoma and its lymph node metastases after iv injection of 10 mg/kg in CD rats. ●——● = primary tumor; ▲- - -▲ = metastases.

nary tumors metastasized from an sc 3LL is significantly greater than in the primary tumor. When the drug assay in the tumor tissue is performed without removing the necrotic areas, the drug concentrations (expressed per g of tissue) in the primary tumor result decreased, as previously reported,[5] and this could be partly responsible for the marked differences observed between drug levels in primary and secondary tumors. However, as indicated by the data on adriamycin concentrations in the viable part of the tumor, other factors, besides the absence of necrosis, should account for the higher drug concentrations observed at the metastases level. This better drug delivery to secondary neoplasms compared to the primary tumors from which they are derived may possibly be explained by the better microvasculature in metastases. It is thought that the response of a given tumor to chemotherapy must be assessed in terms of tumor blood supply besides considering the role of the proliferative state of the tumor cells. In a histologic study Tannock and Steel (26) reported a progressive reduction in the relative vascularity of a tumor as it increases in size. Similar results were obtained by Gullino (27) in tumor perfusion studies. This suggests that large tumors may receive proportionately less blood and therefore less drug on a tumor weight basis; on the other hand, metastases, which are small tumors, should be better vascularized and receive more drug. However, exactly to what extent greater drug sensitivity is related to changes in the relative blood supply and/or the proliferative state of a tumor population needs further investigation.

Other as yet poorly understood factors may also influence the response of metastatic tumor cells to chemotherapy; one is the possibility that during the metastatic process cells are selected with different biochemical characteristics. On the basis of the results described here, we think that the comparatively greater responsiveness of metastases to chemotherapy may involve preferential drug delivery at the metastatic site, thus establishing a possible synergism with the proliferative state of the cell population. The different chemotherapeutic drug levels reached in the primary tumor and in its metastases may explain why the drugs investigated generally revealed in our tumor systems, as indicated by the reported example of adriamycin, a more pronounced inhibitory effect on metastases than on the primary tumor.

LIST OF COMPOUNDS

Adriamycin: NSC-123127; CAS reg. No. 23214-92-8
Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate
Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6

[5]Donelli MG, Broggini M, Colombo T, et al. Importance of the presence of necrosis in studying drug distribution within tumor tissue. Eur J Drug Metab Pharmacokinet. In press.

TABLE 3.—Antitumor and antimetastatic activity of adriamycin on im 3LL in C57BL/6 mice and on im Walker 256 carcinosarcoma in CD rats

Tumor*	Treatment (mg/kg iv)	Primary tumor (g)	Metastases		
			Free animals	Weight/ animal (mg)	No./animal
3LL	—	9.4 ± 0.4	0/10	168.9 ± 35.5	27 ± 2.7
	15 (Day +14)	7.9 ± 0.3†	2/10	1.4 ± 0.5‡	2 ± 0.3‡
Walker 256 carcinosarcoma	—	30.9 ± 1.6	0/6	3800 ± 900	Not determined
	10 (Day +9)	9.2 ± 0.9‡	7/7‡	0	

*Antitumor activity was assessed for 3LL on Day +25 and for Walker 256 carcinosarcoma on Day +15 after tumor transplantation.
†$P < 0.05$.
‡$P < 0.01$.

Hydroxyurea: NSC-32065; CAS reg. No. 127–07–1
6-Mercaptopurine: NSC-755; CAS reg. No. 6112–76–1; purine-6-thiol, monohydrate
Methotrexate: NSC-740; CAS reg. No. 59–05–2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-
1-Methyl-1-nitrosourea: NSC-23909; CAS reg. No. 684–93–5

REFERENCES

1. SCHABEL FM. The use of tumor growth kinetics in planning "curative" chemotherapy of advanced solid tumors. Cancer Res 29:2384–2389, 1969.
2. LASTER WR, JR, MAYO JG, SIMPSON-HERREN L, ET AL. Success and failure in the treatment of solid tumors. II. Kinetic parameters and "cell cure" of moderately advanced carcinoma 755. Cancer Chemother Rep 53:169–188, 1969.
3. DEWYS WD. A quantitative model for the study of the growth and treatment of a tumor and its metastases with correlation between proliferative state and sensitivity to cyclophosphamide. Cancer Res 32:367–373, 1972.
4. STEEL GG, and ADAMS K. Stem-cell survival and tumor control in the Lewis lung carcinoma. Cancer Res 35:1530–1535, 1975.
5. HELLMANN K, SALSBURY AJ, BURRAGE KS, ET AL. Drug-induced inhibition of hematogenously spread metastases. *In* Chemotherapy of Cancer Dissemination and Metastasis (Garattini S, and Franchi G, eds). New York, Raven Press, 1973, pp 355–359.
6. FRANCHI G, MORASCA L, REYERS-DEGLI-INNOCENTI I, ET AL. Triton WR 1339 (TWR), an inhibitor of cancer dissemination and metastases. Eur J Cancer 7:533–544, 1971.
7. DEWYS WD. Studies correlating the growth rate of a tumor and its metastases and providing evidence for tumor-related systemic growth-retarding factors. Cancer Res 32:374–379, 1972.
8. SIMPSON-HERREN L, SANFORD AH, and HOLMQUIST JP. Cell population kinetics of transplanted and metastatic Lewis lung carcinoma. Cell Tissue Kinet 7:349–361, 1974.
9. FRINDEL E, MALAISE EP, ALPEN E, ET AL. Kinetics of cell proliferation of an experimental tumor. Cancer Res 27:1122–1131, 1967.
10. LAIRD AK. Dynamics of tumor growth. Br J Cancer 18:490–502, 1964.
11. MCCREDIE JA, INCH WR, KRUUV J, ET AL. The rate of tumor growth in animals. Growth 29:331–347, 1965.
12. DEWYS WD, GOLDIN A, and MANTEL N. Hematopoietic recovery after large doses of cyclophosphamide: correlation of proliferative state with sensitivity. Cancer Res 30:1692–1697, 1970.
13. BRUCE WR, MEEKER BE, and VALERIOTE FA. Comparison of the sensitivity of normal hematopoietic and transplanted lymphoma colony-forming cells to chemotherapeutic agents administered in vivo. J Natl Cancer Inst 37:233–245, 1966.
14. VALERIOTE F, and VAN PUTTEN L. Proliferation-dependent cytotoxicity of anticancer agents: a review. Cancer Res 35:2619–2630, 1975.
15. SPREAFICO F, and GARATTINI S. Chemotherapy of experimental metastases. *In* The Secondary Spread of Cancer. London, Academic Press. In press, 1977.
16. KARRER K, HUMPHREYS SR, and GOLDIN A. An experimental model for studying factors which influence metastasis of malignant tumors. Int J Cancer 2:213–223, 1967.
17. FRANCHI G, REYERS-DEGLI-INNOCENTI I, ROSSO R, ET AL. Lymph-node metastases after intratibial transplantation of tumors. Int J Cancer 3:765–770, 1968.
18. SCHWARTZ HS. A fluorometric assay for daunomycin and adriamycin in animal tissues. Biochem Med 7:396–404, 1973.
19. CHAKRABARTI SG, and BERNSTEIN IA. Simplified fluorometric method for determination of plasma methotrexate. Clin Chem 15:1157–1161, 1969.
20. FACCHINETTI T, D'INCALCI M, MARTELLI G, ET AL. A simple and sensitive method for the determination of cyclophosphamide by means of a nitrogen phosphorus selective detector (NPSD). Clin Chem, 1977. In press.
21. FRIEDMAN OM, and BOGER E. Colorimetric estimation of nitrogen mustards in aqueous media. Hydrolytic behavior of bis(2-chloroethyl)amine, nor-HN2. Anal Chem 33:906–910, 1961.
22. BOLTON BH, WOODS LA, KAUNG DT, ET AL. A simple method of colorimetric analysis for hydroxyurea (NSC-32065). Cancer Chemother Rep 46:1–5, 1965.
23. FORIST AA. Spectrophotometric determination of streptozotocin. Anal Chem 36:1338–1339, 1964.
24. DONELLI MG, COLOMBO T, FORGIONE A, ET AL. Distribution of 6-mercaptopurine in tumor-bearing animals. Pharmacology 8:311–320, 1972.
25. HOUGHTON PJ, TEW KD, and TAYLOR DM. Some studies of the distribution and effects of cyclophosphamide in normal and neoplastic tissue. Cancer Treat Rep 60:459–464, 1976.
26. TANNOCK IF, and STEEL GG. Quantitative techniques for study of the anatomy and function of small blood vessels in tumors. J Natl Cancer Inst 42:771–782, 1969.
27. GULLINO PM. Organ perfusion and preservation. *In* "In Vitro" Perfusion of Tumors (Norman JC, Folkman J, Hardison WG, et al, eds). New York, Appleton Century Croft, 1968, pp 877–898.

Experimental Chemotherapy With 5-Arylpyrimidine Antifolates: Further Studies on Toxicity and the Responsiveness of Ascitic, Solid, and Intracranial Sarcoma 180 to DDMP and DDMP With Citrovorum Factor[1,2,3]

F. M. Sirotnak,* D. M. Moccio, and D. M. Dorick[4]

SUMMARY

Multiple-dose toxicity of the 5-arylpyrimidine DDMP showed marked seasonal variation and sex dependence in $BD2F_1$ mice. Considerable therapeutic activity against the ascitic, solid, and intracranial forms of Sarcoma 180 was demonstrated. Antitumor effects were highly schedule and dose dependent at a limited number of doses between 16 and 40 mg/kg. Antitumor activity (increase in lifespan) was approximately twofold greater against the ascitic tumor compared to the intracranial tumor at each dose level. The use of citrovorum factor rescue appeared to improve the therapeutic index of DDMP against intracranial Sarcoma 180 on specific multiple-dose schedules. Citrovorum factor rescue did not result in a greater initial antitumor effect of DDMP against the solid Sarcoma 180, but the effect was maintained for a longer period of time by allowing an increase in the number of doses which could be administered without toxicity.

[Cancer Treat Rep 61:1325–1332, 1977]

The high levels of therapeutic effectiveness of methotrexate against some experimental neoplasms and certain forms of human cancer (1–7) appear to bear some relationship to the unique multifactorial role (8) of the target enzyme, dihydrofolate reductase, in macromolecular synthesis. In the case of tumors unresponsive to methotrexate by virtue of an ineffective level of membrane transport (9–15) of this drug, lipophilic agents which are also effective inhibitors of this enzyme, but would gain entry into cells primarily by passive diffusion, may be a useful alternative. Such agents might also provide additional therapeutic advantage when used in conjunction with citrovorum factor rescue, a procedure successfully used to increase the therapeutic index of methotrexate (1,2). Since the natural folate, as with methotrexate, also requires carrier mediation for entry (10,15), therapy with a lipophilic antifolate plus citrovorum factor would involve a different basis for penetration of each. Preliminary documentation of the potential usefulness of this approach during therapy of an ascitic Sarcoma 180 tumor with the 5-arylpyrimidine, DDMP, has been reported (16) in a previous communication from this laboratory. Additional results derived during DDMP therapy of ascitic, solid, and intracranial Sarcoma 180 are now presented here. Other data derived during further studies of DDMP toxicity in mice are also provided.

MATERIALS AND METHODS

Many of the experimental details have been reported earlier (16). DDMP was administered sc 1 day after tumor transplantation (ascitic Sarcoma 180) in the scapular region as the HCl salt in aqueous solution (pH 5.1). A single lot of drug with

[1]Received Dec 27, 1976; revised May 18, 1977; accepted June 2, 1977.

[2]Supported in part by Public Health Service grants CA-08748 and CA-18856 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3]DDMP: NSC-19494; CAS reg. No. 7761-45-7; pyrimidine, 2,4-diamino-5-(3,4-dichlorophenyl)-6-methyl-. Supplied by Wellcome Research Laboratories, Research Triangle Park, NC.

Citrovorum factor: NSC-3590; CAS reg. No. 6055-86-5; glutamic acid, *N*-[*p*-[[(2-amino-5-formyl-5,6,7,8-tetrahydro-4-hydroxy-6-pteridinyl)methyl]amino]benzoyl]-, calcium salt. Supplied by Dr. Harry B. Wood, Jr, Division of Cancer Treatment, NCI, Bethesda, Md.

[4]Laboratory for Molecular Therapeutics, Memorial Sloan-Kettering Cancer Center, New York, NY.

**Reprint requests to:* F. M. Sirotnak, PhD, Laboratory for Molecular Therapeutics, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

no known impurities was used during these studies. Citrovorum factor as the calcium salt in neutral aqueous solution was also administered sc at the same site. Tumor-bearing female $BD2F_1$ (C57BL female/DBA_2 male) mice (20 g) purchased from A. R. Schmidt, Madison, Wis, were selected randomly for control (untreated) and treated groups in each experiment. During therapy against the ascites tumor, mice were inoculated ip with 10^6 tumor cells (0.1 ml). Untreated animals died with a well-developed ascites (average weight, 24–30 g) within 10–13 days. During therapy against the solid tumor, 10^6 tumor cells (0.05 ml) were implanted intradermally in the flank after removing the coat with an electric clipper. At the start of therapy (24 hours after implantation) the average tumor diameter was 2 mm. This increased in untreated controls to 10 mm in 11–14 days. During the total period of the experiment (usually 18 days), the tumor-bearing mice did not develop ascites or exhibit any evidence of metastatic tumor growth. The average tumor diameter in mm is determined from measurements along two axes of the tumor and tumor volume is calculated from the following equation, $V(mm^3) = 4/3\ \pi r^3$. The data are expressed as relative tumor volume (%) by means of the following calculation, $T(V_t - V_0)/C(V_t - V_0) \times 100$, where T = treated group, C = control group, V_0 = volume at the time of treatment, and V_t = volume after treatment. During therapy against the intracranial tumor, 2×10^5 tumor cells (0.02 ml) were implanted to a depth of 3 mm with a 27-gauge needle into the parietal region of the skull. Although implantation actually occurred in the cerebral hemisphere of the brain, the tumor appeared to develop as a meningeal form, since there was no evidence of solid tumor growth. Within a few days mice began to lose weight, developed tremors, and became progressively hypercephalic. Death of untreated animals usually occurred within 10–13 days. Weight at the time of death varied between 10 and 12 g. Evidence that the observed symptoms and eventual death of the animals were due to tumor growth is as follows: (*a*) animals implanted ic with dead cells (induced by storage), tumor cell homogenates, or supernatant from cell suspensions do not develop similar symptoms or die; (*b*) average survival time of mice implanted ic with different amounts of cell suspension showed the expected correlation with cell number; (*c*) intracranial fluid removed from hypercephalic animals shows large numbers of tumor cells and will produce ascites tumors following ip injection in mice; and (*d*) the symptoms and course of the disease are indistinguishable from that seen in mice implanted ic with the G26 glioma. Animals were maintained on Purina laboratory chow and water ad libitum. Deaths due to drug toxicity were generally associated with weight loss of 30%–40%. In the case of drug-treated animals receiving ascites tumors, no ascites were observed at the time of death. In the case of animals receiving tumor cells ic, treated animals dying from drug toxicity were not obviously hypercephalic and could also be distinguished from tumor-induced deaths by weight loss. Data related to single- and multiple-dose intoxication by DDMP have been provided in an early study (17) and in a more recent report (16) from this laboratory.

RESULTS

Multiple-Dose Toxicity of DDMP

The prior report (16) from our laboratory confirmed earlier studies by others (17) and showed that single-dose lethal toxicity of DDMP occurred almost immediately and was related to neurologic effects, but that multiple-dose lethal toxicity of DDMP below the single-dose lethal level was related to effects in the small intestine. It was also shown (16,17) that only DDMP-induced toxicity in the small intestine, as in the case of methotrexate, could be prevented by citrovorum factor. We now report on the marked seasonal variation and sex dependence of multiple-dose DDMP toxicity in $BD2F_1$ mice. Data shown in figure 1 document a twofold variation in tolerance of female mice to DDMP over a period of approximately 2 years (from mid-1974 to mid-1976). Lower tolerances were associated with the period April through September and higher tolerances with the period October through March.

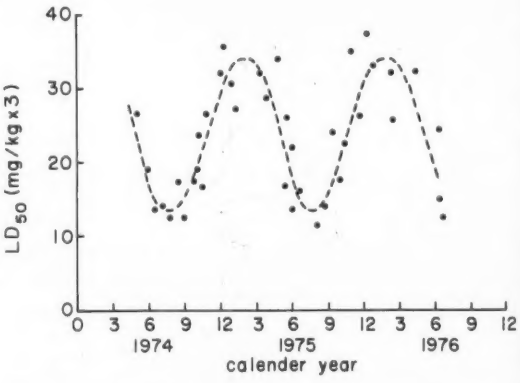


FIGURE 1.—Multiple-dose toxicity of DDMP in $BD2F_1$ female mice. Values for LD50 were calculated from data derived at 8, 16, 24, 32, and 40 mg/kg given for a total of 3 injections q3days to 5 mice each. Each data point represents 1 determination.

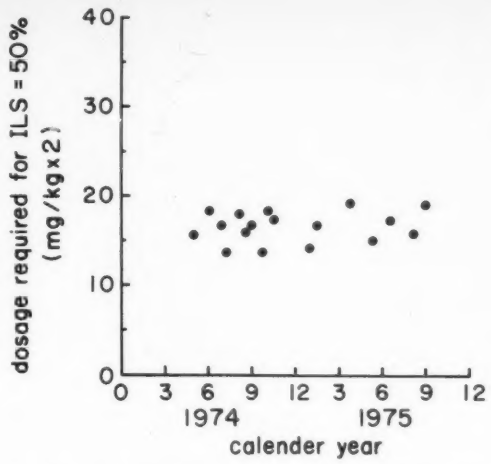


FIGURE 2.—Response of ascites Sarcoma 180 to DDMP. Values were calculated from data derived at 8, 16, 24, 32, and 40 mg/kg given for a total of 2 injections q3days to 5 mice each. Each data point represents 1 determination.

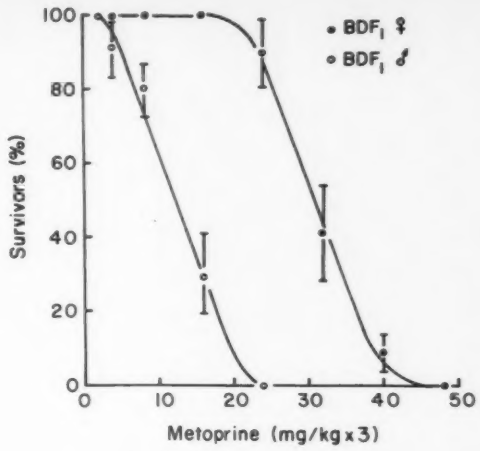


FIGURE 3.—Multiple-dose toxicity of DDMP in male and female $BD2F_1$ mice. Each data point represents the average ± SD of 2 experiments done on different days in which 5 mice received varying doses of DDMP given for a total of 3 doses q3days.

At a dosage of DDMP tolerated during both periods, no apparent variation in the responsiveness of ascites Sarcoma 180 transplanted in female $BD2F_1$ mice could be seen within the total span of the study. An increase in lifespan (ILS) of 50% by two doses q3days consistently required about 16 mg/kg of DDMP (fig 2). Finally, the data given in figure 3 show a threefold decrease in the tolerance of male versus female $BD2F_1$ mice to DDMP. The data presented in the remainder of this paper were obtained during therapy experiments in female $BD2F_1$ mice, which were carried out exclusively within the period of October through April during the years indicated in figure 1.

Responsiveness of Solid Sarcoma 180 to DDMP Alone

In the prior study reported from this laboratory (16), effective regimens of therapy against Sarcoma 180 were obtained in experiments in which dose levels were maximized by the optimization of dosage interval. No therapeutic effect was observed at any dose level using schedules in which DDMP could be given for an unlimited number of doses. Responses were obtained at dose levels of 8–40 mg/kg which could be given for only a limited number of doses. The effect of DDMP against the solid Sarcoma 180 within this dose range given in a limited number of doses at 3-day intervals is shown in table 1. This dosage interval was already found (16) to be optimum and allowed dose-dependent antitumor effects within the range of 16–40 mg/kg during the current study. Inhibition of tumor growth was observed 6 days after a single dose as low as 16 mg/kg and increased to > 60% inhibition at 40 mg/kg. With two doses of DDMP, the antitumor effects were greatly increased, approaching 90% inhibition at 40 mg/kg. Also, effects were maintained for as long as Day 11 (6 days after therapy was stopped). Three doses of DDMP prolonged the tumor inhibition substantially. However, some of the animals given 16 and 24 mg/kg and most of the animals given 32 and 40 mg/kg died of toxicity within 14 days after start of therapy.

Responsiveness of Solid Sarcoma 180 to DDMP With Citrovorum Factor Rescue

The incorporation of delayed citrovorum factor rescue in the previous studies (16) with ascitic Sarcoma 180 permitted the administration of a larger number of doses of DDMP and an increase in the therapeutic index when compared to DDMP alone. A schedule shown (16) to be optimum in which DDMP was given q3days, incorporating citrovorum factor given 24 hours after each DDMP dose, was evaluated against the solid Sarcoma 180 tumor. As before (16), DDMP was given electively, ie, only until the mice exhibited a 15% weight loss. A dose of 6 mg/kg of citrovorum factor shown earlier (16) to be optimum was chosen for use with

TABLE 1.—Response of solid Sarcoma 180 to DDMP

DDMP dose* (mg/kg)	No. of doses	Relative tumor volume (%)†				No. of toxic deaths/total No. of mice
		7 days	11 days	14 days	18 days	
—	—	100	100	100	100	—
16	1	73 ± 7.1	86 ± 12.0	100	100	0/10
	2	31 ± 4.0	38 ± 5.3	58 ± 4.2	75 ± 9.0	0/15
	3	28 ± 2.2	25 ± 2.4	35 ± 3.6	42 ± 3.6	3/15
24	1	58 ± 4.0	80 ± 7.7	91 ± 6.5	100	0/10
	2	25 ± 1.3	35 ± 4.2	48 ± 4.4	68 ± 7.6	0/15
	3	24 ± 2.7	22 ± 3.0	—	—	7/15
32	1	44 ± 3.6	61 ± 7.0	78 ± 8.2	92 ± 8.0	0/10
	2	20 ± 2.1	27 ± 2.3	40 ± 3.8	55 ± 7.5	1/15
	3	18 ± 1.6	18 ± 2.4	—	—	10/15
40	1	38 ± 4.6	52 ± 6.3	69 ± 5.3	84 ± 9.3	0/10
	2	12 ± 1.3	18 ± 1.4	34 ± 3.0	47 ± 5.0	3/15
	3	9 ± 1.1	11 ± 0.9	—	—	10/15

*Administered sc on a schedule of q3days.

†$T(V_t - V_o)/C(V_t - V_o) \times 100 = \%$, $V(mm^3) = {}^4/_3 \pi r^3$. Data shown are average values ± SD obtained with 5 mice in 2 or 3 experiments done on separate days.

TABLE 2.—Response of solid Sarcoma 180 to DDMP with citrovorum factor rescue

DDMP dose* (mg/kg)	Average No. of doses	Citrovorum factor† (mg/kg)	Relative tumor volume (%)‡				No. of toxic deaths/total No. of mice
			7 days	11 days	14 days	18 days	
—	—	—	100	100	100	100	—
16	3–4	—	30 ± 4.0	27 ± 2.3	33 ± 5.0	—	9/15
	4–7	6	32 ± 3.3	37 ± 4.2	41 ± 3.6	38 ± 2.7	0/15
24	3–4	—	23 ± 2.7	20 ± 1.7	20 ± 1.9	—	8/10
	4–6	6	27 ± 3.3	32 ± 3.8	32 ± 2.6	34 ± 4.7	0/15
32	3–4	—	18 ± 2.1	17 ± 1.9	—	—	10/10
	3–6	6	26 ± 3.2	30 ± 3.0	29 ± 0.8	31 ± 1.9	1/15
40	3–4	—	11 ± 1.6	13 ± 1.2	—	—	10/10
	3–5	6	22 ± 2.7	27 ± 4.5	25 ± 2.8	26 ± 1.7	1/15

*Administered sc on a schedule of q3days. Animals were treated until individual weight loss = 15%.

†Given sc 24 hrs after each dose of DDMP.

‡$T(V_t - V_o)/C(V_t - V_o) \times 100 = \%$, $V(mm^3) = {}^4/_3 \pi r^3$. Values shown are averages ± SD obtained with 5 mice in 2 or 3 experiments done on separate days.

DDMP. The data are given in table 2. Citrovorum factor allowed an increase in the average number of doses in the range of 16–40 mg/kg which could be tolerated. The inhibition of tumor growth initially at each dosage of DDMP with citrovorum factor was slightly less than that obtained with the maximum tolerated number of doses of DDMP alone (table 1). However, the inhibition obtained was maintained for a much longer period of time. At 18 days, the difference in tumor volume (T/C) was twice that obtained with DDMP alone for a limited number of doses (table 1). Elective multiple-dose therapy with DDMP alone in the experiment (table 2) was generally unsatisfactory because of toxicity.

Responsiveness of Ascitic and Intracranial Sarcoma 180 to DDMP Alone

Since lipophilic agents effectively penetrate the central nervous system (CNS), an important use for DDMP might be in the treatment of CNS cancer. In the present study, DDMP was evaluated against Sarcoma 180 implanted ip and ic. This offered an opportunity to compare antitumor effects of parenterally (sc) administered DDMP against the same tumor external to and within the CNS compartment. The results of this comparative experiment are presented in table 3. The data shown were derived on a schedule (q3days) previously shown

TABLE 3.—Response of ascitic and intracranial Sarcoma 180 to DDMP given twice with a 3-day interval

DDMP dose* (mg/kg)	Ascites tumor		Intracranial tumor	
	Median survival time† (days)	ILS (%)	Median survival time† (days)	ILS (%)
—	11.8 ± 0.9	—	10.8 ± 1.6	—
16	18.2 ± 1.9	54.2	13.3 ± 1.7	23.2
24	21.2 ± 2.3	79.8	14.5 ± 2.1	34.6
32	22.8 ± 1.7	93.1	16.0 ± 1.9	48.3
40	25.7 ± 3.1	118.6	17.1 ± 3.3	57.7

*Given as a single sc injection 1 day after tumor transplantation.
†Average ± SD of 3 experiments done on separate days with 5 mice per group.

TABLE 4.—Response of intracranial Sarcoma 180 to DDMP given on a schedule of q3days

No. of mice*	DDMP dose (mg/kg)	No. of doses†	Median survival time ± SD (days)	ILS (%)	Average weight in g at death‡ (approximate range)
5 × 4	—		10.6 ± 1.5	—	11.1(10–12)
5 × 2	16	1	10.4 ± 0.9	−2.0	11.4(10–12)
5 × 4		2	13.1 ± 1.8	+22.6	12.4(10–12)
5 × 3		3	15.1 ± 1.5	+39.0	12.2(10–14)
5 × 3		4	14.5 ± 2.1	+36.8	12.8(10–15)
5 × 3		5	12.2 ± 1.3	+15.0	13.7(12–15)
5 × 2	24	1	12.1 ± 1.8	+13.8	10.8(10–13)
5 × 4		2	14.3 ± 1.3	+35.3	11.6(10–13)
5 × 3		3	15.5 ± 1.7	+45.8	12.2(10–14)
5 × 3		4	14.5 ± 2.4	+36.6	13.5(10–15)
5 × 2		5	13.7 ± 1.4	+29.1	13.9(11–15)
5 × 2	32	1	12.5 ± 1.6	+18.8	11.8(10–11)
5 × 4		2	16.6 ± 2.1	+56.2	12.8(10–12)
5 × 4		3	13.8 ± 1.8	+29.6	14.0(10–16)
5 × 3		4	10.3 ± 1.5	−2.5	13.6(12–15)
5 × 3	40	1	14.3 ± 1.8	+34.5	12.2(10–13)
5 × 4		2	17.4 ± 2.6	+64.2	11.6(10–12)
5 × 4		3	10.2 ± 1.3	−4.0	14.0(13–16)
5 × 3		4	9.3 ± 1.2	−12.6	13.7(13–15)

*No. of mice in each experiment × No. of experiments.
†Given sc as a single injection.
‡Initial weight = 20 g.

(16) to allow at least two doses of DDMP at 16, 24, 32, and 40 mg/kg. Antitumor effects obtained in each case were dose related within the total dosage range used. However, the magnitude of the antitumor effect obtained with the intracranial tumor at each dosage was approximately one half of that obtained with the ascites tumor.

Additional data on the responsiveness of intracranial Sarcoma 180 to DDMP alone are given in table 4. When used on a schedule of q3days, the greatest ILSs at 16 or 24 mg/kg were obtained with three doses, but with some evidence of toxicity. On the same schedule, two doses of 32 or 40 mg/kg induced the greatest antitumor effect. Three or more doses at these dosage levels were considerably more toxic.

Responsiveness of Intracranial Sarcoma 180 to DDMP With Citrovorum Factor Rescue

As in the previous study (16) with ascitic Sarcoma 180, an attempt was made to demonstrate an increase in the therapeutic index of DDMP with delayed citrovorum factor rescue. Schedules using DDMP at 2-, 3-, or 4-day intervals were tested with citrovorum factor (6 mg/kg) given 24 hours later.

TABLE 5.—Response of intracranial Sarcoma 180 to DDMP with citrovorum factor

No. of mice*	DDMP dose† (mg/kg)	DDMP dose interval (days)	No. of DDMP doses‡	Citrovorum factor dose§ (mg/kg)	Median survival time ± SD (days)	ILS (%)	Average weight in g at death¶ (approximate range)
5 × 4	—	—	—	—	11.2 ± 1.8	—	11.3(10–12)
5 × 2	16	2	5–7	—	13.4 ± 1.2	19.2	14.1(13–15)
5 × 2			5–8	6	15.5 ± 1.6	38.4	11.6(10–13)
5 × 3	24		5–7	—	14.1 ± 2.1	25.6	13.2(12–15)
5 × 3			6–10	6	16.5 ± 1.3	47.1	11.8(10–14)
5 × 3	32		4–7	—	13.3 ± 1.8	18.5	13.9(12–16)
5 × 3			5–7	6	15.2 ± 1.9	35.7	12.8(10–15)
5 × 3	40		4–6	—	13.5 ± 1.6	19.3	14.2(11–16)
5 × 3			5–7	6	14.7 ± 2.2	31.0	13.5(13–15)
5 × 3	16	3	4–7	—	14.9 ± 1.9	33.2	13.4(11–14)
5 × 3			4–8	6	16.9 ± 1.7	50.8	12.9(10–13)
5 × 3	24		3–5	—	16.2 ± 2.3	44.6	12.6(10–15)
5 × 3			5–8	6	18.1 ± 2.7	61.3	12.3(11–12)
5 × 4	32		3–4	—	17.2 ± 1.9	53.8	13.5(12–15)
5 × 4			3–6	6	21.1 ± 3.0	88.2	11.0(10–15)
5 × 4	40		3–4	—	17.7 ± 2.6	58.0	13.5(12–15)
5 × 4			4–6	6	23.2 ± 3.8	106.7	11.1(10–14)
5 × 2	16	4	4–6	—	15.5 ± 3.2	38.4	12.9(10–13)
5 × 2			4–6	6	14.0 ± 1.9	25.3	11.2(10–13)
5 × 3	24		3–5	—	15.3 ± 2.7	37.0	11.6(10–14)
5 × 3			4–7	6	15.8 ± 1.6	40.6	10.8(10–13)
5 × 3	32		3–5	—	15.9 ± 2.2	51.5	12.3(11–16)
5 × 3			4–6	6	18.2 ± 3.1	62.3	11.2(10–12)
5 × 3	40		3–5	—	17.1 ± 2.4	62.4	12.3(11–14)
5 × 3			4–6	6	20.2 ± 3.4	79.6	11.0(10–12)

*No. of mice in each experiment × No. of experiments.
†Given as a single sc injection.
‡Given until weight loss = 15%.
§1 dose given sc 24 hrs after each DDMP dose.
¶Initial weight = 20 g.

The objective sought was an increase in the number of doses of DDMP which could be tolerated. As in the prior study (16) and in the study shown in table 2, DDMP was given electively, ie, only until a 15% weight loss occurred. The data are given in table 5. The best results were obtained with DDMP alone or with citrovorum factor rescue on a schedule of q3days. The ILS obtained with citrovorum factor rescue was almost twofold better at each dosage of DDMP within the range of 16–40 mg/kg. At the highest DDMP dosage with citrovorum factor rescue, the ILS reached approximately 100%. On a schedule of q2days, citrovorum factor rescue did not appear to prevent lethal toxicity, although a large number of doses could actually be given before a 15% weight loss was observed. On a schedule of q4days, the difference between groups receiving DDMP alone or with citrovorum factor rescue was less than the schedule using DDMP q3days. At the lowest dosages of DDMP, the ILS was actually less with citrovorum factor.

DISCUSSION

These data extend the results of previous studies (16) from this laboratory and elsewhere (18–20) and provide further verification of the antitumor action of DDMP against the Sarcoma 180 tumor. Although no attempt was made to duplicate completely the earlier studies carried out with the Sarcoma 180 ascites tumor model, the results presented here would appear to confirm a prior conclusion, ie, that the best therapeutic effects are obtained with

higher dosages given in a limited number of doses, usually 3–4 days apart. The apparent seasonal fluctuation and sex dependence of multiple-dose toxicity observed during the period of these studies are unexplained.

At dosages equal to or below the maximum tolerated dosage identified for the low tolerance period (April through September), the therapeutic effect against ascitic Sarcoma 180 was equivalent during both high and low tolerance periods. However, in view of the differences in tolerance during each period, a much greater therapeutic effect would be achievable during the high tolerance period (October through March), since the antitumor effect is dose dependent to a dose of 40 mg/kg sc. It should also be noted that the results presented in the earlier report (16) from our laboratory were actually compiled during the period September 1974 through May 1975. The average level of toxicity documented during that study is generally representative of that characteristic of the high tolerance period (October through March) delineated in the 2-year comparison study presented here. Although this information has proven useful during the conduct of these studies, it remains to be determined how relevant this type of data might be to DDMP therapy in other animal species or in patients.

Based on the data presented here which were obtained with the intracranial Sarcoma 180 and the data obtained previously (16) with the ascites Sarcoma 180, the incorporation of citrovorum factor rescue during DDMP therapy does appear to offer some advantage over DDMP alone. However, the increase in therapeutic index observed in both ascites and intracranial models is highly schedule dependent. Optimization requires a careful adjustment of dose interval and level for both DDMP and citrovorum factor. One difference in the results presented here and in the prior report (16) was the ability to more effectively incorporate rescue with higher doses of DDMP, ie, >16 mg/kg. This was possible since experiments were specifically restricted to those periods during which DDMP tolerance was greatest (fig 1). The advantages of citrovorum factor rescue with DDMP therapy in the treatment of solid Sarcoma 180 are less defined. The initial antitumor effect with citrovorum factor rescue is less than that obtained with a tolerated dose schedule of DDMP alone. However, the effect is maintained for a much longer period of time by the larger number of doses made possible by citrovorum factor. It is re-emphasized that as a consequence of the neurologically related, single-dose toxicity, which has a rapid onset (<15 minutes) and is not reversible (16,17) by citrovorum factor, this modality of therapy cannot be applied in mice in exactly the same way as it is used with methotrexate (1–7). In the latter case, the effects of an otherwise lethal dose of methotrexate can be prevented (1,2) by the delayed administration of citrovorum factor, since animals ordinarily would not die until 6–9 days later. The nearly immediate lethality observed with doses of DDMP > 50 mg/kg sc by itself would not allow the use of citrovorum factor in the same way. However, the results of the earlier (16) and current study suggest an alternate approach, ie, the use of citrovorum factor rescue in connection with multiple-dose schedules using DDMP below the single-dose lethal range. This allowed a reduction in the average dose interval and an increase in the total number of doses which could be given without cumulative lethal toxicity. The net result appears to be a substantive increase in the therapeutic index compared to schedules with DDMP alone. Whether or not a similar schedule of DDMP with citrovorum factor will offer some advantage in patients over DDMP alone has not been determined. This will first require a study of the basis and relative dose dependence for single- versus multiple-dose toxicity in man. Finally, it should also be noted that further improvement in the therapy of cancer by the 5-arylpyrimidine class of antifolates might result from an examination of other analogs which exhibited less neurologic toxicity, but were similarly lipophilic and potent inhibitors of dihydrofolate reductase.

The results provided here and in the previous report (16) continue to have significance in view of recent reports (21–23) providing evidence for the possible clinical utility of the general category of 5-arylpyrimidine antifolates in the treatment of human cancer. However, it should also be noted that a very early (24) clinical report provided no evidence of effectiveness.

REFERENCES

1. GOLDIN A, MANTEL N, GREENHOUSE SW, ET AL. Effect of delayed administration of citrovorum factor on the antileukemic effectiveness of aminopterin in mice. Cancer Res 14:43–48, 1954.
2. GOLDIN A, VENDITTI JM, HUMPHREYS SR, ET AL. Factors influencing the specificity of action of an antileukemic agent (aminopterin). Multiple treatment schedules plus delayed administration of citrovorum factor. Cancer Res 15:57–61, 1955.
3. SKIPPER HE, SCHABEL FM, JR, and WILCOX WS. Experimental evaluation of potential anticancer agents. XIII. On the criteria and kinetics associated with "curability" of experimental leukemia. Cancer Chemother Rep 35:1–111, 1964.
4. HERTZ R, LEWIS J, JR, and LIPSETT MB. Five years' experience with the chemotherapy of metastatic choriocarcinoma and related trophoblastic tumors in women. Am J Obstet Gynecol 82:631–640, 1961.

5. CONDIT PT. Chemotherapy of neoplastic disease with folate antagonists. Ann NY Acad Sci 186:475–485, 1971.
6. BERTINO JR, and JOHNS DG. Folate antagonists. *In* Cancer Chemotherapy II. The 22nd Hahnemann Symposium (Brodsky I, and Kahn SB, eds). New York, Grune and Stratton Inc, 1972, pp 9–22.
7. HUTCHISON DJ, ROBINSON DL, MARTIN D, ET AL. Effects of selected cancer chemotherapeutic drugs on the survival times of mice with L1210 leukemia: relative responses of antimetabolite resistant strains. Cancer Res [suppl] 22 (part 2, No. 1):57–72, 1962.
8. WERKHEISER WC. The biochemical, cellular, and pharmacological action and effects of the folic acid antagonists. Cancer Res 23:1277–1285, 1963.
9. SIROTNAK FM, and DONSBACH RC. Further evidence for a basis of selective activity and relative responsiveness during antifolate therapy of murine tumors. Cancer Res 35:1737–1744, 1975.
10. ———. Kinetic correlates of methotrexate transport and therapeutic responsiveness in murine tumors. Cancer Res 36:1151–1158, 1976.
11. KESSEL D, HALL TC, ROBERTS D, ET AL. Uptake as a determinant of methotrexate response in mouse leukemias. Science 150:752–754, 1965.
12. FISCHER GA. Defective transport of amethopterin (methotrexate) as a mechanism of resistance to the antimetabolite in L5178Y leukemic cells. Biochem Pharmacol 11:1233–1234, 1962.
13. SIROTNAK FM, KURITA S, and HUTCHISON DJ. On the nature of a transport alteration determining resistance to amethopterin in the L1210 leukemia. Cancer Res 28:75–80, 1968.
14. SIROTNAK FM, and DONSBACH RC. Differential cell permeability and the basis for selective activity of methotrexate during therapy of the L1210 leukemia. Cancer Res 33:1290–1294, 1973.
15. GOLDMAN ID. The characteristics of membrane transport of amethopterin and the naturally occurring folates. Ann NY Acad Sci 186:400–422, 1971.
16. SIROTNAK FM, DORICK DM, and MOCCIO DM. Experimental chemotherapy with 5-arylpyrimidine antifolates: preliminary studies of toxicity and responsiveness of Sarcoma 180 to DDMP (NSC-19494) and DDMP with citrovorum factor (NSC-3590). Cancer Treat Rep 60:547–553, 1976.
17. HAMILTON L, PHILIPS FS, STERNBERG SS, ET AL. Haematologic effects of certain 2,4-diaminopyrimidines, antagonists of folic acid metabolism. Blood 9:1062–1081, 1954.
18. CLARKE DA, BUCKLEY SM, STERNBERG SS, ET AL. Effects of 2,4-diaminopyrimidines on mouse Sarcoma 180. (Abstr) Cancer Res 12:255, 1952.
19. BURCHENAL JH, GOETCHIUS SK, STOCK CC, ET AL. Diamino dichlorophenyl pyrimidines in mouse leukemia. (Abstr) Cancer Res 12:251, 1952.
20. MEAD JAR, WOOD HB, JR, and GOLDIN A. Relationship of structure to antitumor activity in compounds related to folic acid. Cancer Chemother Rep Part 2, vol 1(2):273–361, 1968.
21. STICKNEY DR, SIMMONS WS, DEANGELIS RL, ET AL. Pharmacokinetics of pyrimethamine (PRM) and 2,4-diamino-5-(3′,4′-dichlorophenyl)-6-methylpyrimidine (DMP) relevant to meningeal leukemia. Proc Am Assoc Cancer Res 14:52, 1973.
22. PRICE LA, GOLDIE JH, and HILL BT. Methodichlorophen as anti-tumour drug. Br Med J 2:20–21, 1975.
23. GEILS GF, SCOTT CW, JR, BAUGH CM, ET AL. Treatment of meningeal leukemia with pyrimethamine. Blood 38:131–137, 1971.
24. MURPHY ML, ELLISON RR, KARNOFSKY DA, ET AL. Clinical effects of the dichloro- and monochlorophenyl analogues of diamino-pyrimidine antagonists of folic acid. J Clin Invest 33:1388–1396, 1954.

Acute Toxicity of Maytansine in F344 Rats [1,2]

G. M. Mugera[3,4] and J. M. Ward[5,6,*]

SUMMARY

The toxicity of maytansine given by sc administration was studied in 5-week-old male F344 rats. The LD50 (14-day) was 0.48 mg/kg. A dose response to drug administration was indicated by body weight changes and diarrhea. A single, acutely toxic dose of maytansine was shown to possess marked activity against dividing cells which was regarded as an important factor in the pathogenesis of acute lesions in tissues with a normal high rate of cell division. Histologically, mitotic figures were observed in many tissues from 6 to 24 hours after drug administration. Subsequently, necrotizing lesions led to atrophic changes in gastrointestinal tract mucosa, thymus, spleen, bone marrow, and testis. Maytansine also induced hemorrhagic lesions in parenchymatous organs and brain and perivascular mononuclear infiltration in the meninges, and chromatolysis and vacuolation of dorsal root ganglion cells, accompanied by clinical signs of ataxia. Ulcerative skin lesions were observed at the sc site of drug administration.

[Cancer Treat Rep 61:1333–1338, 1977]

Maytansine is a novel ansa macrolide isolated from the East African plants, *Maytenus serrata* and *Maytenus buchananii* (1,2), of the genus Celestraceae. Maytansine is an active inhibitor of in vitro growth of cells derived from human carcinoma of the nasopharynx and it shows in vivo activity at a very low concentration over a wide dosage range against murine P388 leukemia and the B16 melanocarcinoma (1,2). The drug inhibits the function of mitotic spindles of growing cultures of L1210 cells and is active in vitro and in vivo at extremely low dose levels, affecting DNA synthesis (3,4). The toxicologic evaluation of maytansine has been performed utilizing beagle dogs and rhesus monkeys (5). In these studies, drug-related toxic effects were enteritis, depletion of lymphoid organs, and bone marrow and liver disorders characterized by fatty infiltration, congestion, and degeneration of hepatocytes. Multiple-dose treatment schedules resulted in nephrosis, degeneration and atrophy of pancreatic acinar cells, and increased mitotic activity in numerous tissues including the pancreas, adrenal cortex, skin, esophagus, stomach, renal pelvis, urinary bladder, ureter, and small and large intestines.

The purpose of this study was to evaluate the acute toxicity of maytansine in rats and compare the results to those observed in other species.

MATERIALS AND METHODS

All drugs were stored at 0°C, and solutions were freshly prepared and injected sc. Solutions of maytansine were prepared by being first dissolved in a few drops of ethanol and then isotonic saline to provide a concentration of 0.1 mg/ml.

Weanling F344 (Charles River Breeding Laboratory, Wilmington, Mass) 4-week-old male rats were obtained. Rats were acclimatized to the laboratory conditions for approximately 5 days prior to initiation of the study when they were 5 weeks old and weighed 80–100 g. They were randomized into groups of ten to give approximately equal mean

[1]Received Jan 5, 1977; revised Apr 4, 1977; accepted Apr 13, 1977.

[2]Maytansine: NSC-153858. Supplied by the Developmental Therapeutics Program, Division of Cancer Treatment (DCT), National Cancer Institute (NCI).

[3]DCT, NCI, Bethesda, Md. Work performed while serving as a Visiting Scientist of the Fogarty International Center.

[4]*Present address:* Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, University of Nairobi, Kabete, Kenya.

[5]DCT, NCI, Bethesda, Md.

[6]We thank Snooky Taylor, Myra Grabin, Ken Pavkov, and Jack Romine for technical assistance and Drs. David M. Young and Felix Mettler for advice and criticism.

[*]*Reprint requests to:* Dr. J. M. Ward, NCI, Delray Bldg, Rm 402, National Institutes of Health, Bethesda, Md 20014.

body weight per experimental group. Rats were housed, five per cage, in filtered polycarbonate cages with automatic water supply and food (NIH mouse and rat ration) ad libitum. The animals were weighed daily and observed closely for deaths and toxic effects during the day following injections and generally three times daily during the 14-day observation period.

Groups of ten rats were administered a single sc injection of one of several doses of the drug to determine the 14-day LD90, LD50, and LD10 according to Finney (6) and Goldstein (7). To study the histogenesis of acute pathologic changes, rats were given LD90 and LD50 doses and two rats of each group were killed 6, 18, 24, 48, 72, and 96 hours after drug administration. Control rats received the vehicle only.

Necropsies were performed on all dead rats and some were killed in extremis. Portions of the liver, kidney, lungs, heart, pancreas, stomach, duodenum, jejunum, ileum, colon, cecum, brain, spinal cord including the dorsal root ganglia, thigh muscle, testis, thymus, bone marrow (femur), spleen, urinary bladder, salivary gland, adrenals, pituitary gland, and skin at the point of injection were collected, fixed in Telly's fixative, and embedded in paraffin. Sections were prepared and stained with hematoxylin and eosin for histologic examination. Selected tissues were stained by the periodic acid Schiff and Von Kossa methods.

RESULTS

Clinical Observations

The lethal doses (with 95% confidence intervals) were: LD10, 0.41 mg/kg (0.38–0.44 mg/kg); LD50, 0.48 mg/kg (0.45–0.50); and LD90, 0.56 mg/kg (0.51–0.60). Maytansine given at dose levels of 0.6–1.0 mg/kg produced a rapid dose-related decrease in body weight (fig 1), diarrhea (sometimes with melena), depression, dyspnea, serous and mucopurulent ocular and nasal exudates, ataxia, hindleg paralysis, and death. Within 2 days after drug administration there was swelling at the site of injection which led to ulceration. Death occurred between the second and fourth day. Dose levels of 0.4–0.6 mg/kg produced loss of body weight, depression, dyspnea, diarrhea, and ataxia. In those animals given 0.50 and 0.58 mg/kg, the ataxia was followed by coma and death. Rats given the lower dose levels recovered rapidly after the fifth day.

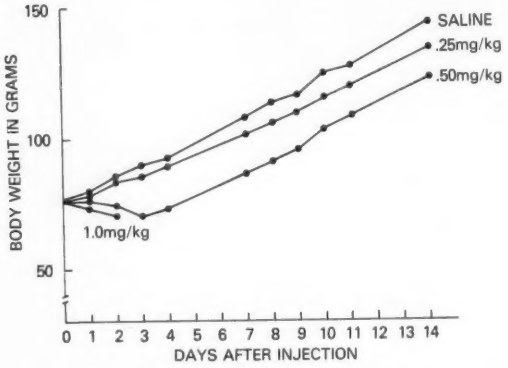


FIGURE 1.—Dose response to a single sc injection of maytansine in male F344 rats, 5 wks old, as measured by changes in body weight.

Pathology

The gross and microscopic lesions were studied from the rats that died or were killed when in extremis and those that were killed at intervals of 6, 18, 24, 72, and 96 hours and 7 and 14 days after drug administration.

The gross and histopathologic changes observed after drug administration depended on the dose and day of death. The most severe changes recorded below were seen in dying rats receiving a ≥ LD50 dose. Grossly, animals killed or dying between 24 and 48 hours after injection had marked congestion in all parenchymatous organs. The stomach and small intestines were distended with excessive fluid and gas. Small hemorrhagic and hyperemic areas were seen in the wall of the duodenum. In rats killed or dying between 72 and 96 hours the spleen and thymus were smaller than normal. The thymus of the control animals was about four times the size of that of the treated animals. In the gastrointestinal tract, the contents were very fluid and frequently contained large quantities of partially digested blood. Occasionally the fluid in the intestine was yellowish in color and mucoid in nature. The wall of the glandular stomach and small intestine was usually transparent and friable. In rats dying from 3 to 7 days after treatment, most organs appeared normal except the gastrointestinal tract where there was excessive mucinous yellowish exudate distending most of the small intestines. The contents of both colon and cecum were fluid. Rats surviving to 14 days had no grossly visible lesions.

Histologic studies at 6 and 18 hours after injection revealed an arrest of mitotic division in the bone marrow, thymus, spleen, testis, gastrointestinal mucosa (fig 2), the basal layer of the skin and hair follicles (fig 3), pancreas, esophagus, pancreatic and salivary gland ducts, renal tubules, renal pelvic uretal and urinary bladder epithelium, adrenal cor-

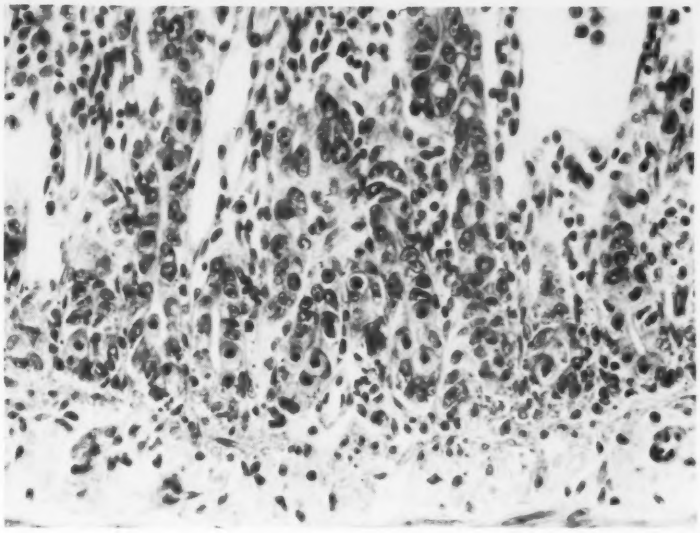


FIGURE 2.—Excessive and abnormal mitosis in crypts of Lieberkuhn of rat injected with 0.75 mg/kg of maytansine and killed 18 hrs after injection (× 350).

FIGURE 3.—Excessive mitotic figures in germinal cells of hair follicle of rat injected with 0.75 mg/kg of maytansine and killed 18 hrs after injection (× 350).

tex, pituitary gland, prostate gland, and liver (hepatocytes and Kupffer cells). The arrest was at mid-phase of cell division (metaphase) as evidenced by an excessive accumulation of such affected cells in the histologic sections of the above organs.

In the gastrointestinal tract at 24–48 hours after injection there was necrosis of the superficial epithelium of the stomach, hemorrhages in the mucosa, and edema, congestion, or hemorrhage in the submucosa. At 24–48 hours, the epithelial cells of the crypts of Lieberkuhn of the small intestine had many eosinophilic droplets and vacuoles in the cytoplasm. The nuclei were large and the nucleoli were prominent. At 48 hours there was pyknosis and karyorhexis of the crypt cell nuclei. At 72–96 hours, there was marked desquamation of the epithelium lining of the crypts, and the lumen of the glands was dilated and filled with necrotic debris. The surviving cells, especially in the crypt, were hyperplastic and enlarged, and several had two or more nuclei. The cells of the villi were shortened and were cuboidal instead of columnar in shape. In the colon and cecum, similar changes were noted and the glands were cystic, while the cysts were filled with necrotic debris and marked submucosal edema was seen. In some areas the epithelial lining had been desquamated but focal areas lined by basophilic and multinucleated hyperplastic cells were seen.

The bone marrow, thymus, and spleen were depleted of hematopoietic cells by 72 hours in dying rats. Surviving rats had regenerative bone marrows. Testicular degeneration and atrophy were seen. Multinucleated giant spermatogenic cells developed (fig 4).

At 24–48 hours after injection, there was vacuolation of the epithelium lining the proximal and distal convoluted tubules in the renal cortex and nuclear pyknosis in the proximal convoluted tubules. In the interstitial spaces of the cortex and medulla, the capillaries were engorged with erythrocytes, and occasional hemorrhages were seen. A few hyaline casts were noted within the lumen of tubules. At 48–96 hours, the lesions were more severe with lymphocytic cells infiltrating the interstitial spaces.

In the central nervous system, there were grossly bilaterally symmetric hemorrhages in or congestion of the meninges and periventricular areas of the brain of rats treated with maytansine at doses of 0.6–1.0 mg/kg. Microscopically, perivascular edema and hemorrhages in the meninges, brain, and periventricular areas were evident, as well as degenerative changes and vacuolation of white and gray matter adjacent to the edematous tissue. In the spinal cord, lesions were observed in the dorsal root ganglion of rats killed on the second day after injection with 1.0 mg/kg. Chromatolysis was seen in ganglion cells as the Nissl substance disappeared,

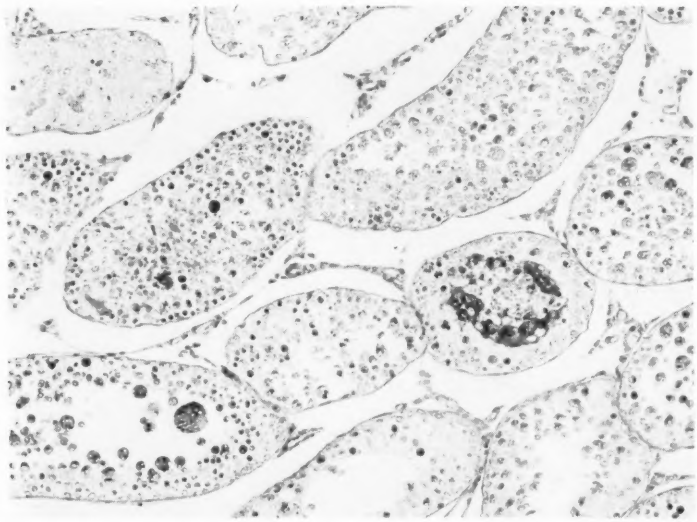


FIGURE 4.—Degenerative and necrotizing changes in seminiferous tubules of rat injected with 0.75 mg/kg of maytansine and killed 72 hrs after injection. Some nuclei of spermatogenic cells are pyknotic and the cytoplasm is vacuolated, while there are many multinucleated giant cells in the lumen of the tubules (× 140).

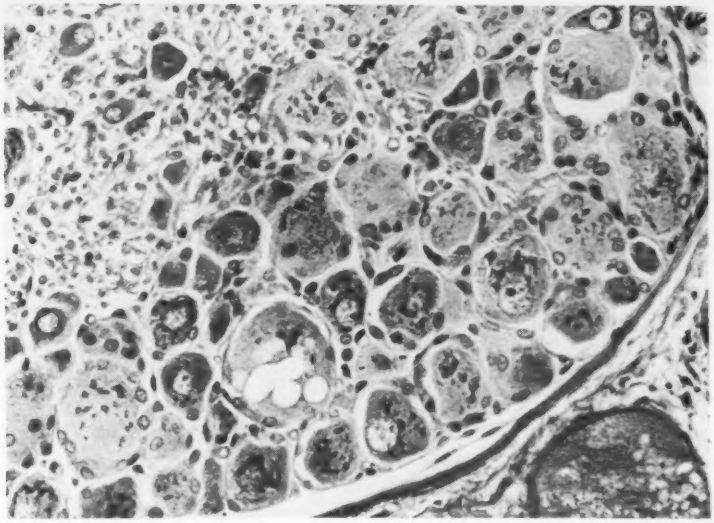


FIGURE 5.—Chromatolysis and vacuolation of the cytoplasm of dorsal root ganglion cells in rat injected with maytansine at 1.0 mg/kg and killed 48 hrs after injection (× 350).

leaving a homogenous eosinophilic ground cytoplasm. Some cells had vacuolation of the cytoplasm, and these vacuoles displaced the nucleus to the periphery of the cell where it assumed a flattened shape (fig 5). Some necrotic cells were seen. In rats treated with maytansine at doses of 0.5 and 0.58 mg/kg, there were no gross lesions in the brain. These rats died between the fifth and eighth day after injection. Histologically, there was marked perivascular and periventricular mononuclear cell infiltration in the meninges and brain. There was also proliferation of the epithelial cells lining the ventricles with perivascular edema and mononucleated cell infiltration. Swelling of the axon cylinders of the sacral nerves was observed. No central nervous system lesions were observed in rats treated with doses of < 0.5 mg/kg.

Skin lesions at the site of injection were necrotizing and ulcerative followed by epithelial acanthosis and dyskeratosis.

In rats that survived after a single dose of maytansine and were killed 14 days later, lesions were not observed except in the liver of rats treated with maytansine at doses from 0.25 to 0.45 mg/kg. Histopathologic evaluation of the livers revealed fatty infiltration of hepatocytes in centrolobular areas and hemosiderin in Kupffer cells.

DISCUSSION

The lesions induced by maytansine in the gastrointestinal tract mucosa, thymus, spleen, bone marrow, and testis were similar to those reported by Peterson et al in rats exposed to dehydroheliotridine, a metabolite of heliotridene-based pyrrolizidine alkaloids (8). Maytansine produced similar lesions as produced by dehydroheliotridine in the hair follicle. Thake et al (5) reported arrest of mitotic cell division in tissue of dogs and monkeys and induction of lesions in gastrointestinal mucosa, hematopoietic system, liver, and kidneys with maytansine. Our studies indicate arrest of mitotic division in cells a few hours after injection in many cell types followed by degenerative and necrotizing lesions. It appears from this study that the acute toxicity of maytansine is associated with interference of cell division followed by degenerative changes and necrosis. The acute gastrointestinal mucosal lesions produced by maytansine were similar to those reported by Bull et al (9) in rats exposed to heliotrine and lasiocarpine and those reported by Hooper (10) in sheep, rats, and mice given lasiocarpine. Van der Watt (11) reported similar lesions in vervet monkeys (*Cercopithecus aethiops*) administered retrorsine.

The lesions in the gastrointestinal mucosa, testis, hematopoietic tissues, and skin are very similar to those produced by exposure to ionizing radiation as reviewed by Upton (12) and those produced by nitrogen mustards (13). Nervous system lesions induced by maytansine are similar to those produced by vincristine (14) and pantothenic acid deficiency in swine (15). The effect of maytansine on the mitotic cells (3,4) appears similar to that of the vinca alkaloids (16,17) and colchicine (18).

REFERENCES

1. KUPCHAN SM, KOMODA Y, COURT WA, ET AL. Maytansine, a novel antileukemic ansa macrolide from *Maytenus ovatus*. J Am Chem Soc 94:1354–1356, 1972.
2. KUPCHAN SM, KOMODA Y, BRANFMAN AR, ET AL. Novel maytansinoids, structural interrelations and requirements for antileukemic activity. J Am Chem Soc 96:3706–3708, 1974.
3. WOLPERT-DEFILIPPES MK, BONO VH, DION RL, ET AL. Initial studies on maytansine-induced metaphase arrest in L1210 murine leukemia cells. Biochem Pharmacol 24:1735–1738, 1975.
4. WOLPERT-DEFILIPPES MK, ADAMSON RH, CYSYK RL, ET AL. Initial studies on the cytotoxic action of maytansine, a novel ansa macrolide. Biochem Pharmacol 24:751–754, 1975.
5. THAKE DC, NAYLOR MW, DENLINGER RH, ET AL. Preclinical toxicologic evaluation of maytansine (NSC-153858) in dogs and monkeys. US Department of Commerce National Technical Service, Feb 1975, PB 244566/AS.
6. FINNEY DJ. Probit Analysis, 3rd ed. London, Cambridge University Press, 1971, 333 pp.
7. GOLDSTEIN A. Biostatistics. New York, MacMillan and Co, 1969, 272 pp.
8. PETERSON JE, SAMUEL A, and JAGO MV. Pathological effects of dehydroheliotridine, a metabolite of heliotridine-based pyrrolizidine alkaloids, in young rats. J Pathol Bacteriol 89:153–171, 1972.
9. BULL LG, CULVENOR CCJ, and DICK AT. *In* The Pyrrolizidine Alkaloids. Amsterdam, North-Holland Publishing Co, 1968, pp 153–156.
10. HOOPER PT. Experimental acute gastrointestinal disease caused by the pyrrolizidine alkaloid. J Comp Pathol 85:341–349, 1975.
11. VAN DER WATT JJ. The acute toxicity of retrorsine, aflatoxin and sterigmatocystin in vervet monkeys. Br J Exp Pathol 51:183–190, 1970.
12. UPTON AC. Biological effects of ionizing radiations. Int Rev Exp Pathol 2:199–240, 1970.
13. LANDING BH, GOLDIN A, NOE HA, ET AL. Systemic pathological effects of nitrogen mustards, and a comparison of toxicity, chemical structure, and cytotoxic effect, with reference to the chemotherapy of tumors. Cancer 2:1055–1066, 1949.
14. QUINTIN LY, THOMAS HM, RICHARD JJ, ET AL. Vincristine neurotoxicity in rodents. Johns Hopkins Med J 121:349–360, 1967.
15. FOLLIS RH, and WINIROBE MM. A comparison of the effects of pyridoxine and pantothenic acid deficiencies on the nervous tissues of swine. J Exp Med 81:539–551, 1945.
16. CARDINALI G, CARDINALI G, and ENEIN MA. Studies on the antimitotic activity of leurocristine (vincristine). Blood 21:102–110. 1963.
17. CUTTS JH. The effects of vincaleukoblastine on dividing cells in vivo. Cancer Res 21:168–172, 1961.
18. STUBBLEFIELD E. DNA synthesis and chromosomal morphology of Chinese hamster cells cultured in media containine *N*-deacetyl-*N*-methylcolchicine (colcemid). *In* Cytogenetics of Cells in Culture (Harris RJC, ed). New York, Academic Press, 1964, vol 2, pp 223–248.

Platinum-Based Polychemotherapy Versus Dianhydrogalactitol in Advanced Non-Small Cell Lung Cancer [1,2]

Robert T. Eagan,* James N. Ingle, Stephen Frytak, Joseph Rubin, Larry K. Kvols, David T. Carr, Douglas T. Coles, and Judith R. O'Fallon [3]

Dianhydrogalactitol (DAG) is the main conversion reaction product of the treatment of dibromodulcitol (DBD) by mild alkali or human serum (1). The dihalohexitols, of which DBD and dibromomannitol are examples, have been regarded primarily as alkylating agents on the basis of their chemical reactivity and their cross-resistance to known alkylating agents (2,3). DBD has shown antitumor activity against squamous cell, small cell, and adenocarcinoma of the lung (4). We have seen one regression in two previously untreated patients with small cell carcinoma and one regression (CNS disease) among four patients who failed prior chemotherapy with cyclophosphamide (C)-based polychemotherapy (unpublished data).[4] Data from our phase I study of DAG (5) suggested a dose of 30 mg/m^2/day iv for 5 consecutive days every 4–5 weeks for phase II trials.

C and adriamycin[5] (A) are among the most active agents in squamous cell, adenocarcinoma, and large cell undifferentiated lung cancer (4). In addition, experiments have shown the combination of C + A to manifest synergism in animal tumor systems (6). *cis*-Dichlorodiammineplatinum(II)[6] (P) is one of a group of coordination compounds of platinum (7) whose effect on DNA resembles that of bifunctional alkylating agents (8). The drug strongly inhibits DNA synthesis with little effect on RNA and protein synthesis (9). The attack on DNA may involve a multiplicity of platinum complex DNA reactions including interstrand and intrastrand cross-linking (10,11) and monodentate and bidentate attack upon various individual DNA bases (12). Rosenberg (9) also suggests that P may enhance the antigenicity of tumor systems allowing the immune system to mount a successful attack upon the tumor.

P has shown activity as a single agent against a variety of human tumors including ovary (13), bladder (14), head and neck (15), and testis and lymphoma (16). The drug has had very little use in lung cancer (16). P is also synergistic with a wide variety of other chemotherapeutic agents including C (17) and A (18). The combination of C + P (19) has shown reasonably good activity against sarcoma, but no responses were noted in three lung cancers treated. The combination of A + P has shown activity against a variety of human tumors (18) including lung cancer.

A prospective randomized clinical trial of DAG versus the combination of C + A + P (CAP-I) in advanced non-small cell lung cancer was carried out to evaluate the two treatment regimens.

MATERIALS AND METHODS

Ninety-nine patients with advanced non-small cell lung cancer were entered in the study. Patients were considered evaluable for the assessment of antitumor activity and survival if they met the eligibility criteria and completed one course of chemotherapy. Of the 99 patients entered in the study, 96 were considered evaluable. Of the three patients not considered evaluable, two receiving CAP-I were considered ineligible because of the absence of measurable or evaluable disease, and one patient receiving DAG failed to complete one course of therapy. Two additional patients receiving CAP-I voluntarily withdrew from the study because of severe nausea and vomiting (one with stable disease and one with tumor regression), but both were considered evaluable. Ninety-two of the 96 evaluable patients had tumor spread beyond one hemithorax and the other four had locally unresectable disease. Patients with normal cardiac and renal status were randomly allocated to treatment with either CAP-I or DAG. Sixteen patients with renal and/or cardiac disease were directly assigned to DAG.

[1]Received Jan 11, 1977; revised Apr 1, 1977; accepted Apr 20, 1977.

[2]Dianhydrogalactitol: NSC-132313; CAS reg. No. 23261-20-3; galactitol, 1,2:5,6-dianhydro-.

[3]Mayo Clinic, Rochester, Minn.

[4]Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

[5]Adriamycin: NSC-123127; CAS reg. No. 23214-92-8.

[6]*cis*-Dichlorodiammineplatinum(II): NSC-119875; CAS reg. No. 15663-27-1.

**Reprint requests to:* Robert T. Eagan, MD, Medical Oncology, Mayo Clinic, Rochester, Minn 55901.

TABLE 1.—Characteristics of the 96 evaluable patients and their primary chemotherapy treatment

Characteristic	Chemotherapy with—					
	DAG			CAP-I		
	Squamous cell	Adenocarcinoma	Large cell	Squamous cell	Adenocarcinoma	Large cell
No. of patients	26	16	13	18	14	9
Median age (yrs)	58	60	62	58	60	56
Sex (M/F)	22/4	15/1	11/2	15/3	10/4	6/3
PS (0–1/2–3)	13/13	12/4	5/8	9/9	11/3	5/4
Indicator disease						
Measurable	12	10	6	9	7	5
Evaluable	14	6	7	9	7	4
Prior therapy of primary tumor						
Surgery	1	0	1	2	2	1
Radiotherapy	2	3	3	3	3	1
Surgery + radiotherapy	1	1	0	0	1	1

Prior to randomization, patients were stratified by cell type, Eastern Cooperative Oncology Group (ECOG) performance score (PS), and measurable versus evaluable disease. The breakdown of the 96 evaluable patients is displayed in table 1. The two groups were quite comparable.

Indicator lesions were considered either measurable or evaluable. Measurable lesions required the presence of clearly defined tumor margins so that the longest perpendicular diameters could be measured. Evaluable lesions were visible and assessable, but not measurable. The 96 patients were evenly divided between the measurable and evaluable categories.

Different criteria were used in assessing tumor response to therapy depending on whether the indicator lesion was measurable or evaluable. A complete regression (CR) required the total disappearance of all tumor and tumor-related abnormalities and applied to both measurable and evaluable disease. A partial regression (PR) applied only to measurable disease and required a $\geq 50\%$ decrease in the product of the longest perpendicular diameters of the indicator lesions. A regression applied only to evaluable disease and required a definite decrease in tumor size agreed upon by two investigators. Stable measurable disease implied a $< 50\%$ reduction or $< 25\%$ increase in the indicator lesion with the appearance of no new lesions. Stable evaluable disease required no definite increase or decrease in tumor size agreed upon by two investigators and no new lesions. Progressive measurable disease required a $\geq 25\%$ increase in size of the indicator lesion or the appearance of any new lesions. Progressive evaluable disease required a definite increase in the size of the indicator lesion or the appearance of a new lesion agreed upon by two investigators. Tumor regressions included CR, PR, and regression responses.

DAG was administered at a dose of 30 mg/m²/day iv for 5 consecutive days with re-treatment scheduled every 4–5 weeks from Day 1. In the CAP-I regimen, the dose of C was 400 mg/m² iv; A, 40 mg/m² iv; and P, 40 mg/m² iv, each given on the first day of each cycle. CAP-I was repeated every 4 weeks. Doses were increased or decreased with the goal being wbc nadirs of 1500–2500/mm³ and/or platelet nadirs of 75,000–100,000/mm³. The dose of P was not increased to > 45 mg/m², and no special diuresis program was employed. P was permanently stopped if the creatinine rose to ≥ 2.0 mg/dl. The maximum cumulative dose of A allowed was 450 mg/m².

RESULTS

Ninety-six patients were evaluable for response to chemotherapy, 55 having received DAG as initial treatment and 41 having received CAP-I. Of these 96 patients, 36 were crossed over to the other drug regimen following progression of disease. Among those 96 patients, 32 tumor regressions were noted in 30 patients, with two patients experiencing regressions with both regimens.

Thirty-one patients with squamous cell carcinoma received treatment with DAG, 26 as initial therapy and five with crossover. Of this group, four of 26 (15%) responded to DAG as initial treatment and none of five responded after failure with CAP-I. In contrast, eight of 18 (44%) patients with squamous cell carcinoma responded to CAP-I as initial treatment as well as an additional four of 13 (31%) after progressing on DAG. Overall, 12 of 31 (39%) patients with squamous cell carcinoma responded to CAP-I.

TABLE 2.—Regression rates according to treatment regimen and histologic type

Histologic type	Treatment	Chemotherapy with—* DAG	CAP-I	P value
Squamous cell	Initial	4/26 (15)	8/18 (44)	0.033
	Crossover	0/5 (0)	4/13 (31)	
	Total	4/31 (13)	12/31 (39)	
Adenocarcinoma	Initial	0/16 (0)	6/14 (43)	0.003
	Crossover	0/2 (0)	2/8 (25)	
	Total	0/18 (0)	8/22 (36)	
Large cell	Initial	2/13 (15)	2/9 (22)	>0.500
	Crossover	0/1 (0)	4/7 (57)	
	Total	2/14 (14)	6/16 (38)	
Cumulative total	Initial	6/55 (11)	16/41 (39)	0.001
	Crossover	0/8 (0)	10/28 (36)	<0.100
	Total	6/62 (10)	26/69 (38)	

*No. of patients with regression; total No. treated (%).

Two patients responded to both regimens (*see* table 2).

Eighteen patients with adenocarcinoma were treated with DAG, 16 as initial treatment and two following disease progression on CAP-I. All 18 failed to respond to DAG. On the other hand, six of 14 (43%) patients with adenocarcinoma responded to CAP-I as initial treatment and two of eight with crossover for a cumulative regression rate of 36% (eight of 22 patients).

Both DAG and CAP-I showed a degree of antitumor activity against large cell carcinoma and were not significantly different. Two of 13 patients responded to DAG as initial treatment and there was no response in the one patient treated with crossover for a total of two of 14 (14%) regressions. CAP-I was slightly, but not significantly, better, with two of nine patients responding to CAP-I as initial treatment and four of seven after failure with DAG. Overall, six of 16 (38%) patients with large cell carcinoma responded to CAP-I.

CAP-I was more effective in inducing a tumor regression in previously untreated patients with squamous cell ($P = 0.03$) and adenocarcinoma ($P = 0.003$) as well as in patients with all three cell types of non-small cell lung cancer who had failed prior chemotherapy with DAG ($P < 0.10$).

The response rates were comparable for patients of all three cell types whether measurable or evaluable disease was present. With DAG as initial treatment, three of 28 patients with measurable disease had PRs as compared with three regressions among 27 patients with evaluable disease. In the group of 41 patients receiving CAP-I as initial treatment, eight of 20 patients with measurable disease responded (one CR and seven PRs) versus eight of 21 with evaluable disease (table 3).

An individual's likelihood for a drug-induced tumor regression was influenced by PS. Among the 55 patients with a PS of 0–1, there were 17 regressions (31%) as compared to five regressions (13%) among the 41 patients with a PS of 2–3 ($P < 0.05$). The trend for higher response rates for patients with better PSs held for both treatments. For DAG there were five regressions (17%) among 30 patients with PSs of 0–1 versus one regression (4%) among 25 patients with PSs of 2–3, and for CAP, there were 12 regressions (48%) among 25 patients with PSs of 0–1 versus four regressions (25%) among 16 patients with PSs of 2–3.

Ninety-six patients were evaluable for determinations of time to progression and survival. Patients who failed to return for an evaluation following the first cycle of chemotherapy were considered to have tumor progression as of Day 35 for the DAG regimen or Day 28 for the CAP-I group. CAP-I was significantly superior to DAG ($P < 0.05$) in delaying time to tumor progression. The median time to progression for CAP-I given as initial treatment was 70 days (range, 9–482+ days) versus 35 days for DAG (range, 13–271 days).

The median survival for all patients was 150 days with no significant difference based on initial drug treatment (table 4). Patients not responding to either treatment survived a median of 98 days as compared with a median survival of 363+ days for patients who responded to initial treatment or a median survival of 453 days for patients responding

TABLE 3.—Responses to initial chemotherapy based on measurable or evaluable indicator disease

Histologic type	Chemotherapy with—* DAG Measurable disease	DAG Evaluable disease	CAP-I Measurable disease	CAP-I Evaluable disease
Squamous cell	2/12	2/14	4/8	4/10
Adenocarcinoma	0/10	0/6	3/7	3/7
Large cell	1/6	1/7	1/5	1/4
Total	3/28	3/27	8/20	8/21

*No. of patients with regression/total No. treated.

TABLE 4.—Median survival

	Chemotherapy with— DAG	CAP-I	Total
All patients			
No. observed	55	41	96
Median survival in days	147*	172*	150
(range)	(13–554+)	(9–552+)	(9–554+)
Responders to initial treatment only			
No. observed	6	16	22
Median survival in days	345	393	363+
(range)	(193–525+)	(99–482+)	(99–525+)
Responders to either initial or crossover treatment			
No. observed	14	16	30
Median survival in days	355+	393+	453*
(range)	(154+–554+)	(99–482+)	(99–554+)
Nonresponders			
No. observed	41	25	66
Median survival in days	84	120	98*
(range)	(13–434)	(9–552+)	(9–552+)

*Determined from Kaplan-Meier survival curve.

to either initial or crossover treatment (table 4). The survival curves for responders and nonresponders are shown in figure 1, the difference being significant at $P < 0.001$. The survival curve and time to progression for the 55 patients initially treated with DAG were calculated both including and excluding the 16 patients directly assigned to DAG because of cardiac and/or renal disease. No demonstrable differences were noted.

TOXICITY

Leukopenia was more common on the CAP-I regimen and thrombocytopenia was more common and severe after DAG therapy. Twenty-seven of 40 patients (68%) with available blood cell count data became leukopenic after the first cycle of CAP-I with a median wbc nadir in those patients of 2700/mm^3 (range, 100–3900/mm^3) on Day 14. Only seven of 40 (18%) patients had thrombocytopenia with a median platelet nadir in those seven patients of 100,000/mm^3 (range, 41,000–120,000/mm^3). After the first cycle of DAG, 44% of evaluable patients (24 of 54) developed leukopenia and 48% (26 of 54) developed thrombocytopenia. In the group of 24 patients developing leukopenia, the median wbc nadir was 2600/mm^3 (range, 100–3900/mm^3) on Day 29. Among the 26 patients experiencing thrombocytopenia, the median platelet nadir was 56,000/mm^3 (range, 2000–117,000/mm^3) on Day 21. The late nadirs seen for DAG were consistent with our phase I data (5). There were no documented bleeding episodes. There was one drug-related septic death (wbc count of 100/mm^3 on Day 9) following CAP-I.

Nausea and vomiting were mild and infrequent following DAG whereas they were frequent and moderately severe following CAP-I. Two patients receiving CAP-I refused further treatment for those reasons.

Renal toxicity following P was not a serious

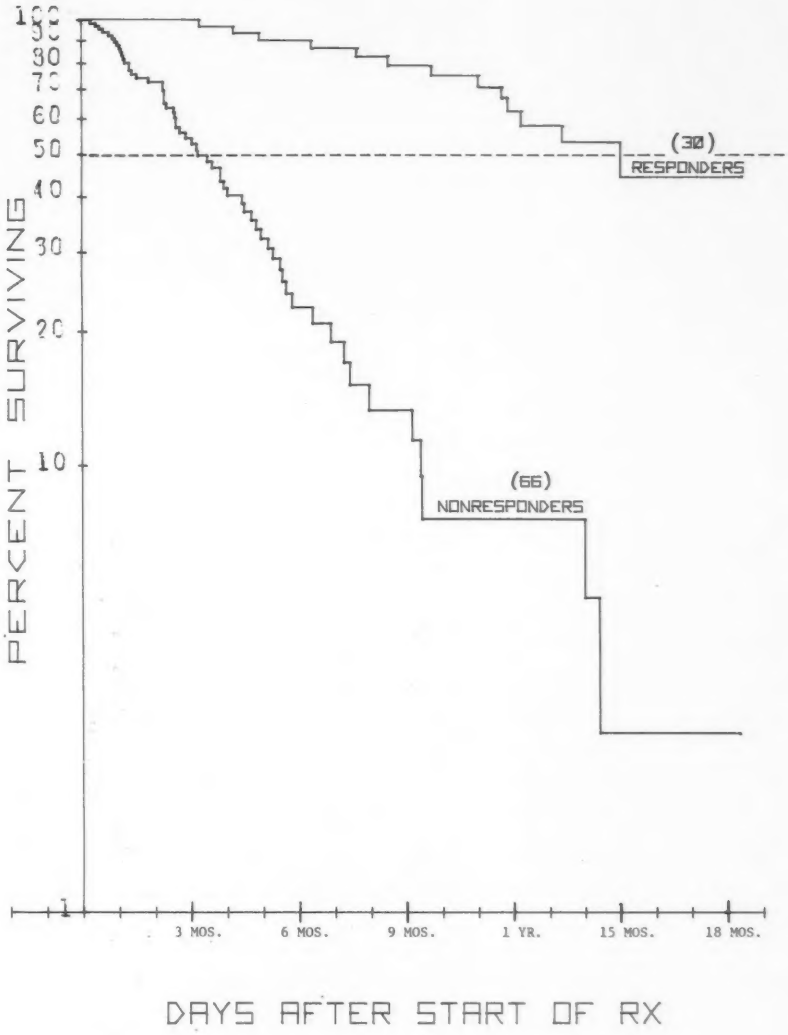


FIGURE 1.—Survival curves for responders and nonresponders to chemotherapy.

clinical problem. Patients were advised to increase their oral fluid intake, but no special diuretic program was employed. Eighteen (31%) of 58 patients with available data increased the serum creatinine above our upper limits of normal (1.2 mg/dl in men) following treatment with CAP-I. The highest serum creatinine recorded 4 weeks following a course of CAP-I was 1.8 mg/dl and the majority were in the 1.3–1.5 mg/dl range. The likelihood of developing an abnormal serum creatinine seemed to be dose-related. Elevated serum creatinines were noted in only 23% (ten of 44) of patients who received a cumulative total dose of P of $\leq$ 240 mg/m^2 versus 57% (eight of 14) of patients receiving $>$ 240 mg/m^2 of the drug (maximum cumulative dose was 516 mg/m^2). All elevated creatinines either stabilized or returned toward normal when P was decreased or discontinued. No clinically evident hearing loss or cardiotoxicity was noted.

DISCUSSION

In the review by Wasserman et al (4) of the clinical chemotherapy of solid tumors, C had regression rates in non-small cell lung cancer of 20%–31% and A, 15%–25%. However, regression rates tend to be much lower in larger randomized series. Edmonson et al (20) reported a 12% response rate for both C and the combination of C + CCNU in adenocarcinoma of the lung. In the report of Edlis et al (21) on the use of C plus heparin in squamous cell lung cancer, only one of 19 patients responded. In their recently reported large series of chemotherapy-resistant patients with non-small cell lung cancer treated with A, Knight et al (22) found response rates of 4%–7%. The combination of C + A has not been studied as extensively as have the individual drugs, but limited data from Lloyd et al (23) and Perlia et al reporting for the ECOG (24) suggest regression rates of $<$ 17%. The combinations of A + P (18) and C + P (19) have been used in too few patients to make any meaningful comments.

The CAP-I regimen had a 39% regression rate in 41 previously untreated patients and a 36% regression in 28 patients previously treated with DAG for an overall regression rate of 38% (26 of 69 patients). This rather high regression rate in patients with non-small cell lung cancer may be the result of a three-way synergism among the drugs in the combination if the lower response rates for the individual drugs used alone are considered. The superior duration of time to progression for CAP-I as compared with DAG and the long survival of the CAP-I responders seem to indicate a considerable degree of antitumor activity for the combination. The lack of significant difference in median survival of the two regimens is almost certainly due to the fact that $<$ 50% of the patients responded to the combination, resulting in no appreciable change in its median survival.

DAG is an interesting chemotherapeutic agent from several standpoints. It seems to have similar or better activity than does C in squamous cell and large cell lung cancer. It also has the ability to cross the blood-brain barrier (25). These two facts, coupled with the lack of alopecia and relative lack of significant nausea and vomiting, make it an agent worthy of further study. Better schedules for its use as a single agent may be developed when the drug's pharmacokinetics are better understood. We are currently exploring its use in the epidermoid cell type of lung cancer, substituting it for C in the CAP-I regimen.

The CAP-I regimen has been used in combination with pulmonary radiotherapy in patients with limited small cell lung cancer (26). It seems to combine well with this other therapeutic modality from the standpoint of acute toxicity. Its use with pulmonary irradiation in patients with unresectable non-small cell lung cancer needs exploration.

REFERENCES

1. INSTITORIS L, HORVATH IP, PETHES G, ET AL. Metabolic pathway of cytostatic dibromohexitols. Cancer Chemother Rep 51:261–270, 1967.
2. KELLNER B, NEMETH L, HORVATH P, ET AL. 1,6-Dibromo-1,6-dideoxydulcitol: a new antitumoral agent. Nature 213:402–403, 1967.
3. CSANGI E, and HALASZ M. Cross-resistance studies on 1,6-dibromo-dideoxy-D-mannitol (DBM)-resistant Yoshida S.C. sarcoma. Br J Cancer 21:353–357, 1967.
4. WASSERMAN TH, COMIS RL, GOLDSMITH M, ET AL. Tabular analysis of clinical chemotherapy of solid tumors. Cancer Chemother Rep Part 3, vol 6(2):399–419, 1975.
5. EAGAN RT, MOERTEL CG, HAHN RG, ET AL. Phase I study of a five-day intermittent schedule for 1,2:5,6-dianhydrogalactitol (NSC-132313). J Natl Cancer Inst 56:179–181, 1976.
6. CORBETT TH, GRISWOLD DP, MAYO JG, ET AL. Cyclophosphamide-adriamycin combination chemotherapy of transplantable murine tumors. Cancer Res 35:1568–1573, 1975.
7. ROSENBERG B, VAN CAMP L, and KRIGAS T. Inhibition of cell division in *Escherichia coli*. Nature 205:698, 1965.
8. HORACEK P, and DROBNIK J. Interaction of *cis*-dichlorodiammineplatinum (II) with DNA. Biochim Biophys Acta 254:341–347, 1971.
9. ROSENBERG B. Possible mechanisms for the antitumor activity of platinum coordination complexes. Cancer Chemother Rep 59:589–598, 1975.
10. ROBERTS JJ, and PASCOE JM. Cross-linking of complementary strands of DNA in mammalian cells by antitumor platinum compounds. Nature 253:282–284, 1975.
11. STONE PJ, KELMAN AD, and SINEX FM. Specific binding of antitumor drug *Cis*-Pt ($NH_3)_2Cl_2$ to DNA rich in guanine and cytosine. Nature 251:736–737, 1974.
12. MANSY S, ROSENBERG B, and THOMSON AJ. Binding of *cis*- and *trans*-dichlorodiammineplatinum (II) to nucleosides. I. Location of binding sites. J Am Chem Soc 95:1633–1638, 1973.

13. WILTSHAW E, and KRONER T. Phase II study of *cis*-dichlorodiammineplatinum(II) (NSC-119875) in advanced adenocarcinoma of the ovary. Cancer Treat Rep 60:55–60, 1976.
14. YAGODA A, WATSON RC, GONZALEZ-VITALE JC, ET AL. *cis*-Dichlorodiammineplatinum(II) in advanced bladder cancer. Cancer Treat Rep 60:917–923, 1976.
15. HAYES D, CVITKOVIC E, GOLBEY R, ET AL. Amelioration of renal toxicity of high dose *cis*-platinum diamminedichloride (CPDD) by mannitol induced diuresis. Proc Am Assoc Cancer Res and ASCO 17:169, 1976.
16. GOTTLIEB JA, and DREWINKO B. Review of the current clinical status of platinum coordination complexes in cancer chemotherapy. Cancer Chemother Rep 59:621–628, 1975.
17. WOODMAN RJ, SIRICA AE, GANG M, ET AL. The enhanced therapeutic effect of *cis*-platinum (II) diamminedichloride against L1210 leukemia when combined with cyclophosphamide or 1,2-bis(3,5-dioxo-piperazine-1-yl) propane or several other antitumor agents. Chemotherapy 18:169–183, 1973.
18. OHNUMA T, HOLLAND JF, and VOGL S. Adriamycin (ADM) and diamminodichloroplatinum (DDP) in combination. Proc Am Assoc Cancer Res and ASCO 16:272, 1975.
19. PIEL IJ, and PERLIA CP. Phase II study of *cis*-dichlorodiammineplatinum(II) (NSC-119875) in combination with cyclophosphamide (NSC-26271) in the treatment of human malignancies. Cancer Chemother Rep 59:995–999, 1975.
20. EDMONSON JH, LAGAKOS SW, SELAWRY OS, ET AL. Cyclophosphamide and CCNU in the treatment of inoperable small cell and adenocarcinoma of the lung. Cancer Treat Rep 60:925–932, 1976.
21. EDLIS HE, GOUDSMIT A, BRINKLEY C, ET AL. Trial of heparin and cyclophosphamide (NSC-26271) in the treatment of lung cancer. Cancer Treat Rep 60:575–578, 1976.
22. KNIGHT EW, LAGAKOS S, STOLBACH L, ET AL. Adriamycin in the treatment of far-advanced lung cancer. Cancer Treat Rep 60:939–941, 1976.
23. LLOYD RE, JONES SE, SALMON SE, ET AL. Combination chemotherapy with adriamycin (NSC-123127) and cyclophosphamide (NSC-26271) for solid tumors: a phase II trial. Cancer Treat Rep 60:77–83, 1976.
24. PERLIA CP, and STOLBACH L, for the ECOG. Adriamycin and Cytoxan in the treatment of inoperable lung cancer. Proc Am Assoc Cancer Res and ASCO 17:27, 1976.
25. LEVIN VA, FREEMAN-DOVE MA, and MAROTEN CE. Dianhydrogalactitol (NSC-132313): pharmacokinetics in normal and tumor-bearing rat brain and antitumor activity against three intracerebral rodent tumors. J Natl Cancer Inst 56:535–539, 1976.
26. EAGAN RT, CARR DT, LEE RE, ET AL. Phase II studies of polychemotherapy regimens in small cell lung cancer. Cancer Treat Rep 61:93–95, 1977.

Tetrahydrouridine: Physiologic Disposition and Effect Upon Deamination of Cytosine Arabinoside in Man [1,2,3]

Willi Kreis,* Thomas M. Woodcock, Clara S. Gordon, and Irwin H. Krakoff [4,5]

SUMMARY

[^{14}C]-tetrahydrouridine (THU), a strong inhibitor of cytidine (CR) deaminase, was, after iv administration, rapidly and quantitatively cleared from the blood with a plasma half-life of about 1 hour. The main pathway of excretion was through the kidneys: most of a dose of 50 mg/kg was excreted within 12 hours and excretion was essentially complete within 48 hours. Oral administration of the same dose revealed absorption of about 10% from the gastrointestinal tract. THU at 10, 25, and 50 mg/kg given 15 minutes before [^{3}H]-cytosine arabinoside (ara-C) at a dose of 0.003 mg/kg produced about a twofold increase in ara-C blood levels at all times measured from 5 minutes to 4 hours, with only slight increases in the half-life of ara-C. A dose-related effect of THU upon the deamination of ara-C was obvious only during the time from 15 minutes to 1 hour after the injection of ^{3}H-ara-C. The inhibitory effect of THU upon CR deaminase was also reflected in a considerably increased ratio of ara-C/uracil arabinoside in the urine.

[Cancer Treat Rep 61:1347–1353, 1977]

A major limitation of the biologic activity of cytosine arabinoside (ara-C), both toxicologic and therapeutic, is its rapid deamination by the enzyme, cytidine (CR) deaminase. This results in very brief persistence of ara-C in blood and tissues. Frequent administration in animals (1) or prolonged iv infusion in man (2) has improved the therapeutic activity of ara-C.

Other approaches to counter the rapid deamination of ara-C in biologic test systems and in man have consisted mainly in the synthesis of ara-C derivatives, such as acylation of the amino group (3) and the 5′-hydroxyl group (4); cyclization of ara-C and 5′-fluoro-ara-C producing cyclocytidine (5) and anhydro-ara-fluoro-C (6); and cyclization of the 5′-ara-CMP yielding 1-β-D-arabinofuranosylcytosine 3′,5′-cyclic monophosphate (7).

Probably the most promising CR deaminase inhibitor is tetrahydrouridine (THU) (8). It produces little toxicity in many species, especially in dogs and monkeys (9)[6] (also Mellett LB, and Philips FS, personal communications), and is an extremely potent competitive inhibitor of pyrimidine nucleoside deaminase (10–14). Due to its structure and special affinity for the deaminase, it was suggested that THU might act as a "transition-state analog" inhibitor (14). In extensive pharmacologic studies in rhesus monkeys, who exhibit a high plasma level of CR deaminase, this enzyme activity was completely inhibited for up to 120 minutes after 12.5–100 mg/kg of THU.[6] In another study, the plasma half-lives for ara-C in the dog, rat, mouse, hamster, and monkey could be extended considerably by pretreatment of these animals with 100 mg/kg of THU (15,16). In

[1]Received Jan 10, 1977; revised Apr 18, 1977; accepted Apr 27, 1977.

[2]Supported in part by Public Health Service grants CA-08748, CA-18557, and CA-18857 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3]Tetrahydrouridine: NSC-112907; CAS reg. No. 5627-05-4; 2(1*H*-pyrimidinone, tetrahydro-4-hydroxy-1-β-D-ribofuranosyl-.

Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride.

[4]Laboratory of Clinical Pharmacology, Memorial Sloan-Kettering Cancer Center, New York, NY.

[5]We are indebted to Lee Anne Carlevarini and Charles Morrow for technical help and to Dr. R. R. Engle, NCI, Bethesda, Md, for supplying [^{3}H]-ara-C.

*_Reprint requests to:_ Dr. Willi Kreis, Memorial Sloan-Kettering Cancer Center, Walker Laboratory, 145 Boston Post Rd, Rye, NY 10580.

[6]Mulligan LT. A comparative study of the metabolism of 1-β-D-arabinofuranosylcytosine in various animal species. PhD thesis, University of Alabama, Birmingham, 1970.

some instances, the enzymatic formation of 5'-ara-CMP from ara-C could be increased significantly when 0.34 mM THU was used in the incubation mixtures containing crude tissue homogenates of some normal and malignant tissue specimens (17).

So far, there is little evidence for increased chemotherapeutic activity of the combination of ara-C and THU over ara-C alone. The studies by Neil et al (18) used L1210 leukemia, which has no CR deaminase activity whatsoever (19,20). Therefore, a beneficial effect of THU in combination with ara-C, both injected ip, would not be expected and was not found. However, a significant improvement over the use of ara-C alone was observed when ara-C and THU were given orally, presumably due to inhibition of CR deaminase of the bacterial flora. In our own laboratory, in four tumors with significant intracellular CR deaminase and sufficient kinase, the combination of ara-C and THU proved beneficial only in the case of Sarcoma 180, and then only when this neoplasm was grown and treated in the ascites but not in the solid tumor form (21).

The present study was undertaken to establish the physiologic disposition of THU in man and to evaluate the possible effect of THU upon the disposition of ara-C in the plasma and urine.

MATERIALS AND METHODS

THU, labeled in the 2 position with ^{14}C (specific activity, 56 millicuries/mmol), was purchased from Nova Chemicals, Rosemead, Calif. The radioactive purity of the material, as evaluated by paper chromatography (PC), was > 93%; the chemical purity was 95%. Unlabeled THU was synthesized by Aerojet, Sacramento, Calif, and was supplied by the NCI, Bethesda, Md. Its chemical purity was 88% ± 3% (batch AJ-53) (according to analysis provided by the NCI). Ara-C, labeled with ^{3}H predominantly in the 5 position, exhibited a specific activity of 8.19 curies/mmol; its radioactive purity was > 99%. Unlabeled ara-C was purchased from The Upjohn Co, Kalamazoo, Mich.

PC was used for the evaluation of the purity of the labeled and unlabeled THU and for the identification of the original compound and its metabolites in urine, plasma, and spinal fluid. Systems and R_f values are given in table 1. For the separation of ara-C and uracil arabinoside (ara-U) the system of Carpenter (22) was used, as described earlier (23). All systems were used at room temperature (about 22°C). Identification of THU was made colorimetrically with a 0.91% solution of Ehrlich's reagent in a concentrated hydrochloric acid:ethanol (1:10) solution, and that of ara-C and ara-U by ultraviolet (UV) light using a UVS-11 lamp (Ultraviolet Products, San Gabriel, Calif). For quantitation of radioactivity derived from [^{14}C]-THU, the technique described earlier was applied (20); for that derived from [^{3}H]-ara-C, the spots were identified in UV light, cut out, and oxidized in a Packard model 306 sample oxidizer (Packard Instruments Co, Downers Grove, Ill). Some areas of minor radioactivity were not identified.

TABLE 1.—R_f values for THU in 2 thin-layer chromatography and 2 PC systems*

System		R_f value
	PC	
1	Ethylacetate:formic acid:water (7:7:1)	0.41
2	Isopropanol:ammonia:water (9:1:2)	0.46
	Thin-layer chromatography	
3	Ethylacetate:formic acid:water (7:7:1)	0.30
4	Isopropanol:ammonia:water (9:1:2)	0.20

*Values are averages of at least 3 experiments. All systems were used at room temperature.

Solutions were prepared for injection by mixing unlabeled THU with the [^{14}C]-THU in sterile 5% dextrose (USP) and filtered through a Millipore filter (0.22 μm). The [^{3}H]-ara-C was mixed with unlabeled ara-C in sterile water (USP) and filtered as above. [^{14}C]-THU was injected iv at a dose of 50 mg/kg in solutions containing 50 mg/ml; for the oral administration of THU, the same [^{14}C]-THU solutions were mixed with equal volumes of orange juice. Oral studies were performed in two patients, both of whom fasted overnight before oral administration. Fifty mg/kg of THU was established to be nontoxic in preliminary clinical studies. The total amount of radioactivity of [^{14}C]-THU administered to each patient was about 200 or 400 microcuries and for [^{3}H]-ara-C was 76 and 147 microcuries. In the combination study of ara-C and THU, THU was administered 15 minutes before ara-C. Each compound was administered as a bolus within 30 seconds.

Blood samples, approximately 10 ml, were collected in heparinized syringes at the intervals indicated in the figures and table 2 for 48–72 hours. Blood samples derived from the combination study of ara-C and THU were collected in syringes containing heparin and extraneous THU at 20 μg/ml. Plasma was prepared by centrifugation of the specimens at 2000 rpm for 10 minutes in an International model Pr-2 Centrifuge (International Equipment Co, Boston, Mass) and stored at –20°C until analysis was performed. For deproteinization of plasma, 2 ml was filtered through a Biogel P-2 (Biorad Laboratories, Richmond, Calif) column (30 × 1.0 cm) and protein and low molecular weight substances were eluted with 0.5 M ammonium for-

mate, pH 5.5–6.0. This procedure was chosen to evaluate possible binding of THU and/or ara-C to plasma constituents. Fractions containing radioactivity were pooled, lyophilized, and analyzed by PC.

Urine samples were stored at −20°C until qualitative analysis was performed with PC. For quantitative analysis, aliquots of plasma, urine, spinal fluid, and homogenized feces were prepared by oxidization in the sample oxidizer. Absorption after oral administration was calculated by dividing the urinary excretion of isotope after oral administration by that after iv administration. Only clear, colorless spinal fluid samples were analyzed. Douglas bags were used for the collection of expired air. CO_2 was trapped in ethanolamine (24). A 5-ml aliquot of each ethanolamine sample was mixed in a scintillation vial with 8 ml of methanol and 9 ml of a toluene solution containing 4 g of 2,5-diphenyloxazole and 100 mg of 1,4-bis-2-(5-phenyloxazolyl)benzene per 1000 ml of toluene and counted in the scintillation counter. Due to external standardization and built-in absolute activity analyzer, corrections for quenching were necessary only for the noncombusted samples. The values in table 2 and the figures are the mean values for two determinations.

RESULTS

Six patients with far-advanced nonresectable cancer who had been treated previously with other drugs, but not with ara-C, and from whom a specific informed consent had been obtained to participate in this study, received 50 mg/kg of THU iv or orally (two patients each) or ara-C in combination with THU iv (two patients).

Table 2 summarizes the routes of excretion after iv and oral administration of [^{14}C]-THU alone. The iv administration of [^{14}C]-THU resulted in rapid excretion of radioactivity predominantly in the urine. Within 12 hours, 78% and 80.9% of the administered radioactivity were excreted in the urine, and within 24 hours, the values were 93% and 90.8%. Thereafter, only minor amounts of radioactivity were recovered: after 48 hours the values were 93.5% and 94.1%. In the feces, after iv injection, only minor amounts of radioactivity were recovered (0.17% and 0.19%) over the 48-hour collection time. Expired air contained approximately 0.2% and 0.1% of the injected radioactivity over 2 and 4 hours respectively. Clearly, the bulk of the radioactivity was excreted in the urine (fig 1). After oral administration, 11.4% and 6.5% were excreted in the urine over 48 hours. After oral administration, the excretion apparently must have continued beyond the collection time, although at a very reduced level (fig 1). The feces contained 52.3% and 27.6% within 24 and 72 hours.

TABLE 2.—Time of collection and routes of excretion of ^{14}C-THU-derived radioactivity following iv and oral administration of 50 mg/kg of ^{14}C-THU

	Time of collection (hrs)	% administered radioactivity
Iv administration		
Patient 1		
Urine	48	94.1
Feces	72	0.017
Expired CO_2	(6)	0.21
Total		94.3
Patient 2		
Urine	48	93.5
Feces	48	0.19
Expired CO_2	(6)	0.14
Total		93.8
Oral administration		
Patient 3		
Urine	48	11.4
Feces	24	52.3
Expired CO_2	(4)	0.36
Total		64.1
Patient 4		
Urine	48	6.5
Feces	72	27.6
Expired CO_2		Not done
Total		34.9

The calculated average absorption from the gastrointestinal tract after oral administration amounted to about 9.9%.

The qualitative analysis of the urinary products after iv administration revealed only the original compound in the acid PC system, whereas in the alkaline PC system, besides the predominant starting material, a second compound was found in minor amounts. It is possible that conversion from THU monomer to THU dimer takes place under the influence of the alkali of the second PC system. No further attempts were made to clarify the unidentified material.

The qualitative analysis of the urinary products, after oral administration, revealed three and four labeled products in the acid and basic PC systems respectively. Varying amounts of the different excretion products (including the original THU) were found over the period of the urine collection. Again, no attempt was made to analyze the different products nor to evaluate their effect upon CR deaminase. In the strong acidic system (system 1, table 1), both THU and THU dimer have identical

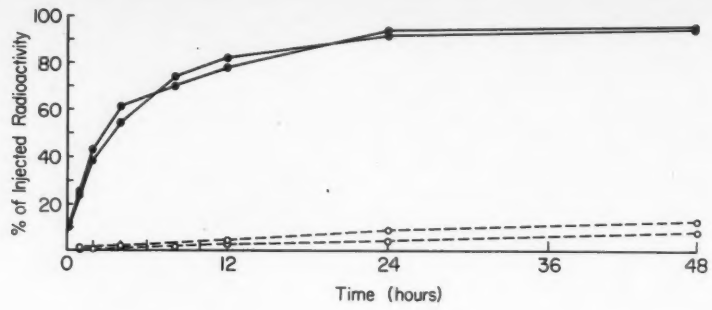


FIGURE 1.—Cumulative urinary excretion of [^{14}C]-THU-derived radioactivity following iv (●——●) or oral (○---○) administration of 50 mg/kg of [^{14}C]-THU. Abscissa: time after administration of THU. Ordinate: cumulative excretion in % of administered radioactivity.

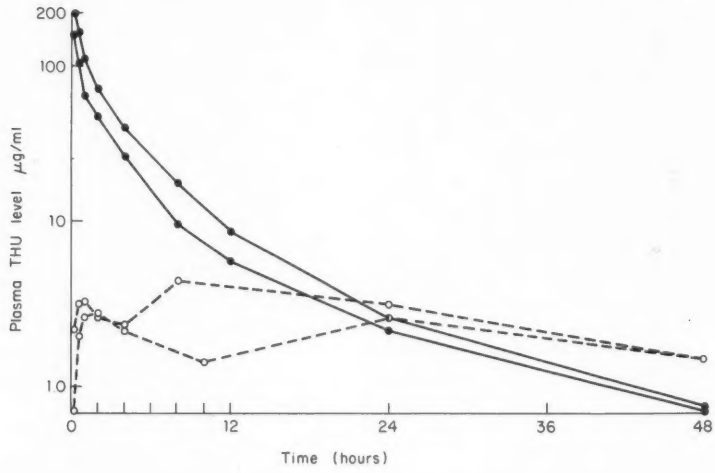


FIGURE 2.—THU equivalents in plasma after single iv or oral administration of 50 mg/kg of [^{14}C]-THU. ●——● = iv administration; ○---○ = oral administration. Abscissa: time after administration of THU. Ordinate: µg THU equivalents. For procedure *see* text.

R_f values of 0.41. In the basic system (system 2, table 1), THU has an R_f value of 0.46. The THU dimer (kindly supplied by Dr. J. Moravek, Moravek Biochemicals, City of Industry, Calif) in system 2 exhibited two pink spots (staining with Ehrlich's reagent) with R_f values of 0.46 and 0.62. It is likely that the material migrating with an R_f value of 0.46 represents THU monomer, and the one with an R_f value of 0.62 represents THU dimer. The latter seems to be more stable in alkali than in the acidic system. With regard to the patient study, these results indicate that the dimer of THU could quite possibly account for the presence of a "metabolite" in system 2 but probably not in system 1.

Plasma levels, after iv injection (fig 2), revealed maximum levels of 197 µg/ml of THU equivalents (based on plasma radioactivity) at 15 minutes, with a rapid decrease within 60 minutes, resulting in a half-life of 50 and 60 minutes for the first part of the exponential curve and about 3½ hours for the second part of the curve. Complete separation of radioactivity from plasma proteins was easily and quantitatively achieved with the filtration on Bio-rad P-2. Also, a comparison of whole blood, plasma,

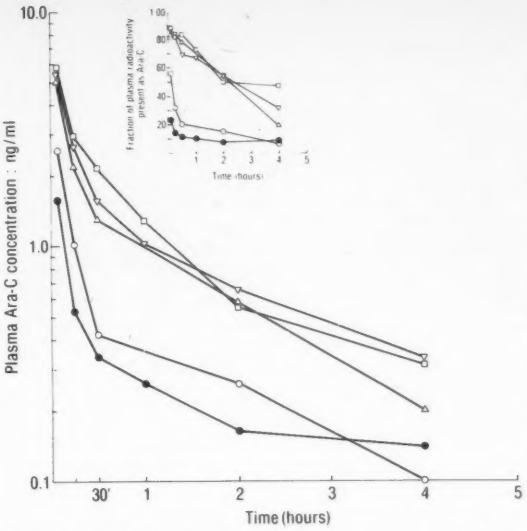


FIGURE 3.—Influence of iv administered THU at 10, 25, and 50 mg/kg upon the plasma levels of unchanged ara-C after iv injection of 0.003 mg/kg of [^{3}H]-ara-C to 2 patients. Controls: ○——○ = patient 1; ●——●——● = patient 2. △——△ = pretreatment of patient 1 with 10 mg/kg of THU; ▽——▽ = pretreatment of patient 2 with 25 mg/kg of THU; □——□ = 50 mg/kg of THU. Abscissa: time after injection. Ordinate: ng of ara-C/ml of plasma. Insert: fractions of plasma radioactivity representing ara-C as a function of time. Same symbols as main figure. Abscissa: time after injection. Ordinate: fraction of ara-C out of total plasma [^{3}H]-ara-C-derived radioactivity.

and red blood cells indicates that all of the radioactivity exists in material easily removable from formed particles and plasma. No qualitative analyses were performed on these plasmas.

Plasma levels after oral administration (fig 2) revealed maximal levels after 7 and 2 hours respectively for patients 3 and 4, reaching levels of 4.4 and 2.8 μg/ml of THU equivalents. In one patient the 24-hour plasma level after oral administration was slightly higher than after iv administration, probably due to a protracted absorption from the gastrointestinal tract. No qualitative analysis was performed on the plasma samples collected after the oral administration of the material.

Cerebrospinal fluid was obtained by lumbar puncture in only one patient after iv administration. The values were very low at 1 and 24 hours after the administration of the radioactive material (1.11 and 1.89 μg/ml of THU equivalents respectively). At these times the corresponding plasma levels were 60.90 and 2.25 μg/ml of THU equivalents. Saliva was collected at hourly intervals for 5 hours following iv administration of [^{14}C]-THU. The ratios of saliva/plasma were low and never exceeded 0.1.

Studies of the combination of ara-C and THU revealed striking effects of THU upon the patterns of ara-C in blood and urine. As demonstrated in figure 3, [^{3}H]-ara-C alone, at a dose of 0.003 mg/kg iv, produced plasma ara-C levels of 2.56 and 1.67 ng/ml at 5 minutes after administration. At 30 minutes

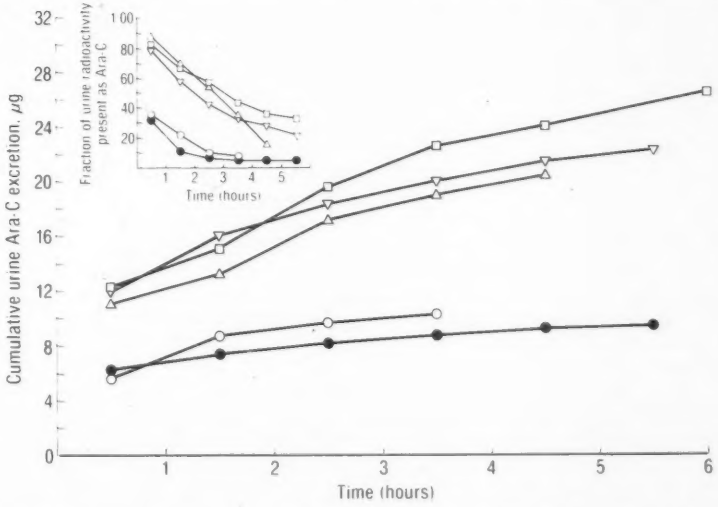


FIGURE 4.—Influence of iv administered THU at 10,, 25, and 50 mg/kg of THU upon the urine levels of unchanged ara-C after iv injection of 0.003 mg/kg of [^{3}H]-ara-C to 2 patients. For symbols *see* fig 3. Abscissa: time after injection. Ordinate: cumulative urine excretion of ara-C. Insert: fractions of urine radioactivity representing ara-C as a function of time. Same symbols as main figure. Abscissa: time after injection. Ordinate: fraction of ara-C out of total urine [^{3}H]-ara-C-derived radioactivity.

both controls were at comparable levels (0.42 and 0.34 ng/ml). The half-lives for both controls are 11.4 and 9.8 minutes for the first phase and 1 hour and 25 minutes for the second phase. The combination of ara-C with 10 mg/kg of THU produced initial ara-C blood levels of 5.1 ng/ml of plasma at 5 minutes, decreasing with an initial half-life of 13.1 minutes in 30 minutes to a level of 1.3 ng/ml; at 4 hours after the administration, the ara-C levels were reduced to levels comparable to the controls. Twenty-five and 50 mg/kg of THU had effects upon the ara-C levels similar to the effect of 10 mg/kg. Plasma levels at 5 minutes after the injection were 5.1 and 5.6 ng/ml, and at 30 minutes after the injection these values were 1.6 and 2.2 ng/ml. At 4 hours the ara-C levels were low but still about three times higher than the controls. The corresponding initial half-lives were 14.0 and 19.1 minutes. When the fraction of radioactivity representing ara-C in plasma was plotted (insert, fig 3), it was obvious that the controls exhibited an exponential decrease, whereas with 10 or 25 mg/kg of THU, the decrease resembled more a reaction of zero order. This is also true for 50 mg/kg of THU up to 2 hours, but not when the ara-C percentage was included at 4 hours after the injection. The influence of THU upon the excretion of ara-C in the urine is shown in figure 4. There was, clearly, a marked increase of ara-C excretion, with a decrease in excretion of ara-U. Although 10 and 25 mg/kg of THU have approximately the same inhibitory effect upon the deamination of ara-C to ara-U, the effect of 50 mg/kg of THU provided a still more significant inhibition of ara-C deamination.

DISCUSSION

Iv administration of THU at 50 mg/kg led to maximal plasma levels 15 minutes after the injection with a fast, exponential decrease over 24 hours. The initial half-lives, 55 and 60 minutes, are considerably higher than the half-lives reported for ara-C (12 minutes) (25), a fact essential for achieving any beneficial effect upon the deamination of ara-C. Excretion of THU occurred almost exclusively through the urine, the bulk of the material being excreted within 12 hours and nearly all within 48 hours.

Oral administration led to a relatively poor and slow absorption into the bloodstream; the maximal plasma levels after oral administration are at best one fortieth of the maximal levels obtained after iv injection. The total urinary excretion of radioactivity amounted to only about one tenth of the excretion after iv injection, which implies an absorption from the gastrointestinal tract of about 10%. The urinary excretion still continued, however, to a low degree beyond 48 hours. After iv injection, the predominant product of excretion in the urine was unchanged THU. After oral administration, four substances were identified in the urine; it is possible that some of the material is represented by the dimer of THU. Metabolites or breakdown products, however, cannot be excluded at this time.

The influence of THU upon ara-C levels is expressed predominantly as elevated ara-C levels, amounting to a doubling of the initial ara-C levels and protracted elevation of unchanged ara-C over 4 hours. A dose-related effect of THU upon the inhibition of deamination is obvious only in the time between 15 minutes and 1 hour, and an increase of the initial half-lives (first phase) is not marked but suggested.

Due to the extremely low dose of ara-C administered in these studies, conclusions with respect to an influence of the half-life of therapeutic doses of ara-C under concomitant treatment with THU can only be drawn after further studies at different levels of ara-C dosages. However, preliminary data from the phase I study of THU and ara-C indicate that 10 mg/kg of THU potentiates the toxicity of ara-C by about tenfold and 25 mg/kg by about 20-fold (26).

These preliminary data presented do not yet allow a conclusion as to whether a saturation of all the body CR deaminases has been achieved or not, or whether such a state is achievable at all. To demonstrate total deaminase inhibition, plasma levels and urines would have to exhibit no deamination of ara-C.

Since increased inhibition of ara-C deamination was observed with the use of 50 mg/kg of THU compared to 25 mg/kg, it is possible that saturation of deaminase binding sites has not yet been achieved. This might be due to limited transport across cell membranes metabolism of THU into biologically ineffective derivatives or other restrictive mechanisms.

Studies are in progress to further elaborate the extracellular and intracellular effects of THU upon the metabolism of ara-C.

REFERENCES

1. SKIPPER HE, SCHABEL FM, JR, and WILCOX WS. Experimental evaluation of potential anticancer agents. XXI. Scheduling of arabinosylcytosine to take advantage of its S-phase specificity against leukemic cells. Cancer Chemother Rep 51:125–141, 1967.
2. FREI E, HO DHW, BODEY GP, ET AL. Pharmacologic and cytokinetic studies of arabinosylcytosine. *In* Verifying Concepts of Leukemia (Dutcher RM, and Chieco-Bianchi L, eds). Bibl Haematol 39:1085–1097, 1973.

3. WEMPEN I, MILLER N, FALCO EA, ET AL. Nucleosides. XLVII. Syntheses of some N^4-substituted derivatives of 1-β-D-arabinofuranosylcytosine and 5-fluorocytosine. J Med Chem 11:144–148, 1968.
4. NEIL GL, BUSKIRK HH, MOXLEY TE, ET AL. Biochemical and pharmacological studies with 1-β-D-arabinofuranosylcytosine-5'-adamantoate (NSC-117614). A depot form of cytarabine. Biochem Pharmacol 20:3295–3308, 1971.
5. HOSHI A, KANZAWA F, KURETANI K, ET AL. 2,2'-*O*-cyclocytidine, an antitumor cytidine analog resistant to cytidine deaminase. Gann 62:145–146, 1971.
6. FOX JJ, FALCO EA, WEMPEN I, ET AL. Oral and parenteral activity of 2,2'-anhydro-1-β-D-arabinofuranosyl-5-fluorocytosine against both intraperitoneally and intracerebrally inoculated mouse leukemia. Cancer Res 32:2269–2272, 1972.
7. KREIS W, and WECHTER WJ. Studies on drug resistance. IV. Synthesis and biological activities of 1-β-D-arabinofuranosylcytosine 3',5'-cyclic monophosphate and a derivative. Res Commun Chem Pathol Pharmacol 4:631–639, 1972.
8. HANZE AR. Nucleic acids. IV. The catalytic reduction of pyrimidine nucleosides (human liver deaminase inhibitors). J Am Chem Soc 89:6720–6725, 1967.
9. GOLDENTHAL EI, COOKSON KM, GEIL RG, ET AL. Preclinical toxicologic evaluation of tetrahydrouridine (NSC-112907) in beagle dogs and rhesus monkeys. Cancer Chemother Rep Part 3, vol 5(1):15–16, 1974.
10. MULLIGAN LT, JR, and MELLETT LB. Comparative metabolism of cytosine arabinoside and inhibition of deamination by tetrahydrouridine. Pharmacologist 10:167, 1968.
11. CAMIENER GW. Studies of the enzymatic deamination of ara-cytidine. V. Inhibition in vitro and in vivo by tetrahydrouridine and other reduced pyrimidine nucleosides. Biochem Pharmacol 17:1981–1991, 1968.
12. HO DHW. Inhibition of 1-β-D-arabinofuranosylcytosine phosphorylation in human liver by tetrahydrouridine. Biochem Pharmacol 21:2936–2940, 1972.
13. CHABNER BA, JOHNS DG, COLEMAN CN, ET AL. Purification and properties of cytidine deaminase from normal and leukemic granulocytes. J Clin Invest 53:922–931, 1974.
14. WENTWORTH DF, and WOLFENDEN R. On the interaction of 3,4,5,6-tetrahydrouridine with human liver cytidine deaminase. Biochemistry 14:5099–5105, 1975.
15. MULLIGAN LT, and MELLETT LB. Inhibition of arabinosylcytosine deamination by tetrahydrouridine in the monkey. Pharmacologist 12:221, 1970.
16. EL DAREER SM, MELLETT LB, WHITE V, ET AL. Inhibition of deamination of ^{3}H-arabinosylcytosine (ara-C) by tetrahydrouridine (H_4U) in BDF_1 mice. Pharmacologist 16:210, 1974.
17. FURNER RL, MELLETT LB, and HERREN TC. Phosphorylation of 1-β-D-arabinofuranosylcytosine (ara-C) in the presence of tetrahydrouridine (H_4U): kinetics of ara-C metabolism by solid tumors in vitro. Pharmacologist 16:210, 1974.
18. NEIL GL, MOXLEY TE, and MANAK RC. Enhancement by tetrahydrouridine of 1-β-D-arabinofuranosylcytosine (cytarabine) oral activity in L1210 leukemic mice. Cancer Res 30:2166–2172, 1970.
19. DURHAM JP, and IVES DH. Deoxycytidine kinase. I. Distribution in normal and neoplastic tissues and interrelationships of deoxycytidine and 1-β-D-arabinofuranosylcytosine phosphorylation. Mol Pharmacol 5:358–375, 1969.
20. DOLLINGER MR, BURCHENAL JH, KREIS W, ET AL. Analogs of 1-β-D-arabinofuranosylcytosine. Studies on mechanism of action of Burkitt's cell culture and mouse leukemia and in vitro deamination studies. Biochem Pharmacol 16:689–706, 1967.
21. KREIS W, and HESSION C. Enzyme, distribution and combination studies with 1-β-D-arabinofuranosylcytosine (ara-C) plus tetrahydrouridine (THU) in 4 animal neoplasms. Proc Am Assoc Cancer Res and ASCO 17:83, 1976.
22. CARPENTER DC. Paper chromatography of nucleic acid derivatives. Anal Chem 24:1203–1204, 1952.
23. UCHIDA K, and KREIS W. Studies on drug resistance. I. Distribution of 1-β-D-arabinofuranosylcytosine, cytidine and deoxycytidine in mice bearing ara-C sensitive and resistant P815 neoplasms. Biochem Pharmacol 18:1115–1128, 1969.
24. KALBERER F, KREIS W, and RUTSCHMANN J. The fate of psilocin in the rat. Biochem Pharmacol 11:261–269, 1962.
25. HO DHW, and FREI E, III. Clinical pharmacology of 1-β-D-arabinofuranosylcytosine. Clin Pharmacol Ther 12:944–954, 1971.
26. WONG P, CURRIE V, MACKEY R, ET AL. Phase I study of tetrahydrouridine (THU) with arabinosyl cytosine (ara-C). Proc Am Assoc Cancer Res and ASCO 18:196, 1977.

Combinations of Tetrahydrouridine and Cytosine Arabinoside in Mouse Tumors [1,2,3]

Willi Kreis,* Catherine Hession, Angela Soricelli, and Kevin Scully [4,5]

SUMMARY

Thirteen experimental mouse neoplasms were tested for cytidine (CR)-deaminase and deoxycytidine (dCR)-kinase levels. Four neoplasms, Sarcoma T241, Adenocarcinoma E0771, Lewis lung carcinoma (LL), and Sarcoma 180 Japan (S180J), considered to have high deaminase and sufficient dCR-kinase activities, were tested in vivo for combination chemotherapy with cytosine arabinoside (ara-C) and the CR-deaminase inhibitor, tetrahydrouridine (THU). THU did not significantly improve the growth inhibition of ara-C in a wide range of combinations in T241, E0771, LL, and the solid form of S180J, but more than doubled the survival time of the S180J ascites-bearing animals. Toxicity in the form of weight loss and toxic deaths was observed in some but not all groups, especially at high dosages of ara-C and THU. Tissue distribution of [^{3}H]-ara-C and [^{14}C]-THU in T241-bearing mice revealed an accelerated clearance of ara-C-derived radioactivity under the influence of THU in the tumor and five host tissues, but not in the small intestines. With the exception of the small intestines, clearance of THU-derived radioactivity was faster in all tissues studied compared to the clearance of [^{3}H]-ara-C-derived radioactivity. Intracellular CR-deaminase levels were inhibited significantly, ie, dose dependent, in tumor and host kidney after a single ip injection of THU to E0771-bearing mice. In the solid S180J, with or without simultaneous ip administration of THU, [^{3}H]-ara-C was not converted to 5′-di- and tri-phosphates at all. In mice bearing the ascites form of S180J, [^{3}H]-ara-C was extensively converted to ara-C 5′-di- and tri-phosphates. THU increased both overall ara-C-derived radioactivity and the relative amounts of ara-C 5′-di- and tri-phosphates.

[Cancer Treat Rep 61:1355–1364, 1977]

Inactivity of cytosine arabinoside (ara-C) in human leukemias has been attributed to its deamination to the biologically inactive uracil arabinoside (ara-U) (1–6) and also to insufficient production of ara-C 5′-phosphates (1,7–11).

Resistance to ara-C in experimental tumors has been attributed chiefly to the lack of phosphorylating enzymes (1,12–16), although other mechanisms have also been described (17,18). Nucleoside deaminase (cytidine [CR]/ara-C deaminase) does not play a major role in the resistance of most, but probably not all, animal tumors investigated (1,2,12,19).

Efforts to circumvent or inhibit the deamination of ara-C, pursued in many laboratories, have resulted in the synthesis of analogs of deoxycytidine (dCR) or ara-C which are not substrates for nucleoside deaminases (20–28). The most useful deaminase inhibitor to date is tetrahydrouridine (THU) (29–33). When given alone, even at high dosages, it shows low toxicity in many species, especially in

[1]Received Feb 20, 1976; revised Apr 18, 1977; accepted Apr 25, 1977.

[2]Supported in part by Public Health Service grants CA-08748 and CA-18557 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3]Tetrahydrouridine: NSC-112907; CAS reg. No. 5627-05-4; 2(1*H*)-pyrimidinone, tetrahydro-4-hydroxy-1-β-D-ribofuranosyl-.

Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride.

[4]Laboratory of Biochemical Pharmacology, Memorial Sloan-Kettering Cancer Center, New York, NY.

[5]We are indebted to Dr. Dorris J. Hutchison for her continuous interest and to Dr. R. R. Engle, NCI, Bethesda, Md, for supplying [^{3}H]-ara-C.

**Reprint requests to:* Dr. W. Kreis, Laboratory of Biochemical Pharmacology, Memorial Sloan-Kettering Cancer Center, 145 Boston Post Rd, Rye, NY 10580.

dogs and rhesus monkeys (34). It is a very efficient competitive inhibitor for CR/ara-C deaminase (23,30–33,35,36), and its reported K_i varies from 10^{-4} to 10^{-5} (23) to 2.9–5.4 × 10^{-8} M (36,37).

At present, there is little evidence for increased chemotherapeutic activity with the combination of ara-C + THU versus ara-C alone in experimental test systems. In vivo studies by Neil et al (38) were carried out using the L1210 mouse neoplasm. Since L1210 cells do not exhibit CR/ara-C deaminase activity (19,20), a direct beneficial effect upon the target cells was not expected or found (38). Apparently, THU had no significant activating effect upon dCR/ara-C kinase nor, as demonstrated, was a slight increase in the plasma half-life of ara-C (38) beneficial. In in vivo tests with two experimental lymphomas and a mammary adenocarcinoma, Cooper and Greer (39) found twofold to threefold increases in the incorporation of dCR into DNA under the influence of THU. They attributed the increases exclusively to dCR-deaminase inhibition but did not evaluate dCR-deaminase activities in these tissues.

None of the 14 experimental tumor systems reported in the literature (1,12,19,39) approached, to any comparable value, the high deaminase levels found in human leukemic cells (1,3,6,36). It was, therefore, desirable to screen further experimental tumor systems for neoplasms with high deaminase and significant kinase levels in order to select test systems more comparable to human leukemias.

MATERIALS AND METHODS

A series of solid and ascites tumors (mast cell tumor [P815]; mast cell tumor resistant to ara-C [P815/ara-C]; Lewis lung carcinoma [LL]; Sarcoma T241; Adenocarcinoma E0771; Sarcoma 180 Japan [S180J]; Ridgway osteogenic sarcoma; Mecca lymphosarcoma; Carcinoma 19-B; B16 melanoma, pigmented; B16 melanoma, nonpigmented; methylcholanthrene-induced tumor A; and spontaneous leukemia in AKR mice), presently maintained by serial transplantation or stored in liquid nitrogen at the Sloan-Kettering Institute, were implanted sc by trocar in appropriate strains of mice. Well-developed tumors were removed, cooled to 0°C, minced, and homogenized in four volumes (wt/vol) of cold 0.25 M sucrose solution. The cellular debris was removed by centrifugation at 1000 × g (2000 rpm) for 15 minutes and further centrifuged at 100,000 × g for 60 minutes. For the deaminase assays both of the supernatants were either used directly or after freezing for no longer than 1 day. For the kinase assays, the 100,000 × g supernatant was used instantly or stored as mentioned for the deaminase studies.

No attempts were made in this study to check the stability of these enzymes over a prolonged time.

Deaminase Assay

Each assay contained 200 μl of modified Krebs-Ringer (40) buffer, pH 7.4, 50–200 μl of 1000 or 100,000 × g supernatant from 20% tissue homogenates, and 4 μmols of CR or ara-C in 50 μl. Incubation at 37°C varied from 30 to 60 minutes. The reaction was terminated by the addition of 200 μl of cold 10% trichloroacetic acid (TCA). Precipitated protein was removed by centrifugation at 1000 × g for 20 minutes in an International PR2 centrifuge. The total supernatant was added to the Conway microdiffusion dish (Brunswick Laboratories, St. Louis, Mo). Controls contained all ingredients (including TCA) but were not incubated. The amount of ammonia released was determined according to the method of Roth et al (41).

Kinase Assay

Each assay tube contained adenosine triphosphate, 3 μmols; magnesium chloride, 3 μmols; 3-phosphoglyceric acid, 2.5 μmols; and tris-HCl buffer, pH 8.0, 20 μmols in 100 μl. Substrate was either ^{14}C-labeled 2-dCR, 5.1 nmols, with a specific activity of 1.31 millicuries/mmol, or [^{3}H]-ara-C, 5.0 nmols, with a specific activity of 30 millicuries/mmol in 50 μl. In one group of assays, THU to a concentration of 0.15 μM was added. Between 50 and 100 μl of the 100,000 × g supernatant from 20% tissue homogenate containing 0.5–1.2 mg of protein was added. A suitable amount of sucrose (0.25 M) was substituted for enzyme in the controls. The final volume was adjusted to 250 μl with tris buffer when necessary. After incubation for 30 minutes at 37°C, the reaction was stopped by immersing the tubes in an ice bath. Duplicate 50 μl samples were spotted on DEAE filter discs (Whatman DE81, 2.5 cm) and processed as reported earlier (12). The dried filters were counted in a Packard (Downers Grove, Ill) Tricarb scintillation spectrophotometer, model 3380, in a diotol or toluene-permablend (Packard) scintillation mixture. ^{14}C-dCR and [^{3}H]-ara-C were obtained from Schwarz-Mann (Orangeburg, NY) and the National Cancer Institute (NCI) (Bethesda, Md) respectively.

For chemotherapeutic assays, female BDF_1 mice weighing 18–24 g were inoculated sc with tumor by trocar (Day 0). After 24 hours (Day 1), treatment with five to ten daily injections was initiated. Ara-C was dissolved in 0.9% saline at a concentration of 2–10 mg/ml and 0.2 ml was injected ip per 20 g of mouse. THU (chemical purity, 88% ± 3%), supplied

by Dr. Harry B. Wood (NCI), was dissolved in 2–100 mg/ml of saline. When injected in combination with ara-C, one half of the dosage of THU was administered ip ½ hour before ara-C, and the remainder was injected simultaneously with ara-C but contralateral to the injection site of ara-C. The weights of the mice were recorded daily, and the sizes of the tumors were evaluated on Days 8 and 15 after initiation of treatment. The longest and widest diameters of the tumors were measured and added, and the T/C ratio was calculated.

$$\text{Acceptance value} = \frac{\text{lower 99.5 confidence limits}}{\text{average value}} \text{ for the tumor}$$

growth index of control tumors. [Values supplied by G. Tarnowski.]

Enzyme assays after administration of THU alone were done by using well-developed E0771 (usually 9–11 days after inoculation) bilaterally grown in BDF_1 mice. THU solutions were prepared as described for the chemotherapy experiments. Either ½ or 2 hours after injection both kidneys and both tumors were removed. Preparation of the homogenates and enzyme assays were the same as described above.

For the tissue distribution studies in T241-bearing BDF_1 mice, [^{3}H]-ara-C (radioactive purity, > 99%; specific activity, 0.289 millicurie/mmol) was injected at a concentration of 60 mg/kg ip alone or simultaneously with [^{14}C]-THU (radioactive purity, > 93%; specific activity, 0.024 millicurie/mmol) at a concentration of 1000 mg/kg ip to groups of three to four mice. At the times indicated in figure 1, the mice were killed by cervical dislocation, the blood was collected from the retro-orbital plexus, and the tissues were removed immediately, cooled, weighed, and stored in a frozen state until quantitative analysis was performed with a model 306 sample oxidizer (Packard). The resulting ^{14}C- or ^{3}H-containing vials were then counted in a Packard model 3380 scintillation spectrophotometer with external standardization.

The quantitative evaluation of radioactivity in blood or tumor tissue of mice carrying solid or ascites S180J was done as previously described after such tumor-bearing BDF_1 mice had been injected with [^{3}H]-ara-C (radioactive purity, > 99%; specific activity, 6.08 millicuries/mmol) at 15 mg/kg ip alone or in combination with THU at 1000 mg/kg ip either in unlabeled or ^{14}C-labeled material (specific activity, 0.037 millicurie/mmol). Acid-soluble intracellular ara-C and ara-C metabolites were extracted with 0.32 N perchloric acid (PCA) from all homogenates. The PCA extracts were neutralized with 2 N KOH, and the reaction products were

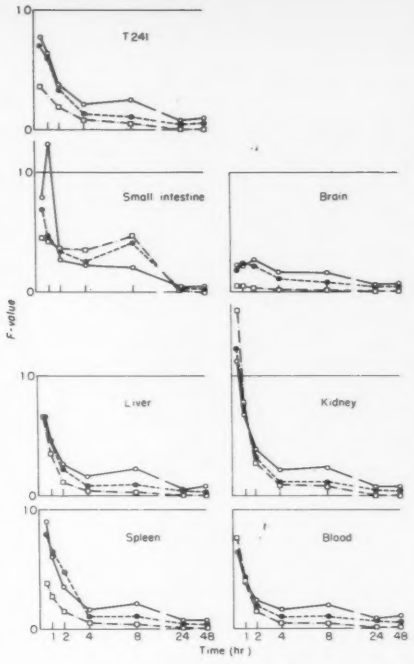


FIGURE 1.—Distribution of radioactivity after an ip injection of 60 mg/kg of [^{3}H]-ara-C alone or in combination with 1000 mg/kg of [^{14}C]-THU. [^{3}H]-ara-C-derived radioactivity in the treatment with ara-C alone (○——○) or combined with THU (●—●). [^{14}C]-THU-derived radioactivity in the combination experiment (□—·—□). Abscissa: time tissues removed after injection; ordinate: linear plotting of F value. For definition of F value and methods *see* text. Each point represents the mean value of 3–4 combined samples.

analyzed by high-pressure liquid chromatography (42,43) or by paper chromatography (Whatman No. 3MM, system: *n*-propanol, tetrahydrofurfuryl alcohol, citrate buffer at pH 5.66 [2:1:1]) (12,44). Location and quantitation of the spots were done as described earlier (45). Protein was assayed according to the method of Lowry et al (46).

The concentration of radioactivity in the distribution studies was expressed by F values. For the studies with T241-bearing mice, F is defined as:

$$F = \frac{\text{specific activity (cpm/g tissue, wet weight)} \times \text{body weight (g)}}{\text{total cpm administered}}.$$

For the studies in S180J tumors (ascites and solid), F is defined as:

$$F = \frac{\text{specific activity (cpm/g protein)} \times \text{body weight (g)}}{\text{total cpm administered}}.$$

TABLE 1.—Deaminase and kinase assays of tumor homogenates

Tumor mouse strain*	CR deaminase† (nmol/mg/hr)		dCR kinase† (nmol/mg/hr)	
	1000 × *g* supernatant (1st determination)	100,000 × *g* supernatant (1st determination)	100,000 × *g* supernatant (1st determination)	
			–THU	+THU
LL solid	127.6 ± 26.7	179.2 ± 28.5	1.40 ± 0.08	1.41 ± 0.10
T241 solid	187.0 ± 9.5	192.2 ± 10.9	0.89 ± 0.07	0.88 ± 0.02
E0771 solid	174.7 ± 7.5	253.1 ± 8.8	1.04 ± 0.05	1.01 ± 0.04
P815 ascites	< 5	< 5	12.88 ± 0.42	
P815/ara-C	< 5	< 5	0.38 ± 0.04	
S180J solid	47.6 ± 3.8	30.0 ± 4.5	1.00 ± 0.05	0.89 ± 0.04
S180J ascites	52.8 ± 3.1	86.8 ± 11.3	2.33 ± 0.13	2.07 ± 0.13
Mouse kidney	950 ± 44.7	1618.04 ± 108.2	0.19 ± 0.03	

*Inoculated either sc or ip. All tumors grown in BDF_1 mice.

†Nos. indicate mean values of 5 assays of a representative experiment. Limit of detection for CR deaminase, 5 nmols/mg/hr. Limit of detection for dCR kinase, 0.015 nmol/mg/hr.

RESULTS

Of seven experimental mouse tumors in table 1, only four showed significant levels of deaminase in both the 1000 and 100,000 × *g* supernatants. The highest CR-deaminase value was found in E0771, with values of 174.7 and 253.1 nmols/mg/hour for the two homogenates, followed by T241, LL, S180J (ascites), and S180J (solid). Seven other experimental tumors tested showed deaminase levels below or only slightly above the limit of sensitivity of the method. In comparison, P815 mouse neoplasm exhibited no detectable CR-deaminase activity, whereas BDF_1 mouse kidney showed deaminase activity about five to six times higher than E0771 tumor homogenate. Although such high CR-deaminase levels, as described for acute lymphocytic leukemia, acute myelocytic leukemia (AML), and chronic myelocytic leukemia (1,3,6), were not attained in these mouse neoplasms, the levels found in both LL and E0771 were 100 times higher than the highest levels reported by Durham and Ives (19) in a series of nine experimental tumor systems; the levels are probably still higher than the highest values reported by Furner and Mellett (47) in their series of 13 experimental tumor systems. For AML, the CR-deaminase levels of 190, 418 (6), and 3.5–23 nmols/mg/hour (ara-C as substrate) (1) were reported. A repeat of the deaminase assays in two cases revealed that, after 2 weeks of freezing the extract, the loss of enzyme activity was about 20%. dCR-kinase assays in the 100,000 × *g* supernatant revealed the highest activity in the P815 ascites cells followed, with much lower activities, by S180J (ascites), LL, E0771, S180J (solid), T241, and the ara-C-resistant P815/ara-C ascites tumor. No significant difference was seen whether or not the reaction tube contained the deaminase inhibitor THU. These kinase levels compare favorably with the levels reported for AML (0.87 nmol/mg/hour [ara-C substrate] [1], and 7.5 nmols/mg/hour [dCR substrate] [6]). No attempt was made to study the time-dependent stability of the dCR kinase in these crude extracts or to evaluate the enzyme activity depending on the age of the tumor.

In therapeutic experiments with E0771, T241, and LL, as evaluated by the diameter of the tumors, ara-C alone showed a slightly significant effect in E0771 on Day 15 and in T241 on Days 8 and 15 after treatment with 100 mg/kg. In both groups severe toxicity was also observed. Due to the lack of acceptance values for LL, the effect of ara-C alone cannot be evaluated adequately.

THU, in combination with ara-C, was evaluated in a wide range of dosages in these three solid tumors. In E0771, ara-C given ip at 20, 40, 60, and 100 mg/kg + THU given ip from 10 to 1000 mg/kg decreased rather than increased the chemotherapeutic activity over the treatment with ara-C alone. Toxicity (in the form of early death and weight loss) was observed in the combinations of 60 or 100 mg/kg of ara-C + 100 or 1000 mg/kg of THU. Toxicity was even more pronounced when ara-C was administered ip and THU was administered sc. In T241, ara-C evaluated at 20, 40, 50, 60, and 100 mg/kg ip in combination with ip THU from 10 to 1000 mg/kg showed a slight beneficial effect and severe toxicity at 60 and 100 mg/kg of ara-C + 1000 mg/kg of THU. In LL tested with the combination of ara-C ip at 20, 60, and 100 mg/kg + THU ip at 20–1000 mg/kg, no improvement over the treatment with ara-C alone was observed but increased toxicity was observed with high-dose schedules of ara-C + THU. THU given alone at the dose of 1000 mg/kg

TABLE 2.—Effectiveness of THU alone and in combination with ara-C in solid tumor and ascites forms of S180J in BDF, mice

Treatment and dose (mg/kg)		Solid tumor (5 mice/group)					Ascites (10 animals/group)		
		Mean tumor diameter and SD		Treated/control		Weight change on Day 6 (g/mouse)	Mean survival time and SD	Treated/control (% CLS)	Weight change on Day 6 (g/mouse)
Ara-C	THU	Day 8	Day 15	Day 8	Day 15				
Control		75.8 ±16	100.6 ±18.6				15.5 ±4.5	0	+2.1
15	—	63.5 ±11.8	96.4 ±37.7	0.84	0.96	−1.0	20.2 ±9.0	+30	+4.1
60	—	43.7 ±7.4	78.1* ±24.3	0.58	0.78	−3.4	15.1 ±8.7	−3	−1.0
100	—						13.0 ±6.8	−16	0
15	10	73.0 ±20.0	98.5 ±26.1	0.96	0.98	−1.2	20.7 ±6.9	+34	+1.1
15	100	74.0 ±29.0	104.6 ±36.9	0.97	1.04	−1.0	34.1† ±15.2	+120	+1.0
15	1000	65.3 ±10.3	88.0 ±16.2	0.86	0.88	−0.6	24.8 ±8.3	+60	−1.0
60	100	51.8 ±11.6	89.3 ±18.8	0.68	0.89	−1.2			
60	1000	52.5 ±20.7	97.3* ±31.0	0.69	0.97	−2.0	8.6 ±2.0	−44	−1.5
100	10						6.6 ±5.7	−57	−3.1
100	1000						7.2 ±1.0	−54	−3.1
	1000	78.4 ±17.88	96.6 ±18.45	0.94	0.94	+0.2	13	−7	+1.9
Acceptance values‡				0.6	0.7		Acceptance value	+31	

*1 death.
†2 60-day survivors.
‡In CD, mice, as evaluated by G. Tarnowski (personal communication) (48).

to E0771-, T241-, and LL-bearing mice, showed neither toxic nor tumor-inhibiting effects. Attempts to convert these three solid tumors into ascites form have been unsuccessful.

In S180J, a striking difference in the response to chemotherapy of the solid versus the ascites form was noticed (table 2). In the solid tumor form, a marginal therapeutic effect was obtained only with 60 mg/kg of ara-C alone, and no benefit was observed in the combination treatment with THU. Toxicity in the form of weight loss was reduced under the influence of THU.

In the ascites form, a significantly beneficial therapeutic effect was obvious with the combination of 15 mg/kg of ara-C + 100 mg/kg of THU (change in lifespan [CLS] = +120%) and less so with the combination of 15 mg/kg of ara-C + 1000 mg/kg of THU (CLS = +60%), while ara-C alone at 15 mg/kg ip produced only marginal therapeutic effect. When 60 or 100 mg/kg of ara-C (which when given alone showed no therapeutic effect and little toxicity) was combined with THU (60 mg/kg of ara-C + 1000 mg/kg of THU and 100 mg/kg of ara-C + 10 or 1000 mg/kg of THU), considerably shortened survival times and severe weight losses were observed. Long-term survivors (two) were found only in the combination of ara-C at 15 mg/kg + THU at 100 mg/kg.

THU alone at 1000 mg/kg had no therapeutic or toxic effects in either the solid or ascites form of S180J.

Distribution experiments in T241-bearing BDF, mice, performed to possibly explain the lack of any beneficial effect of THU upon the effect of ara-C in the therapeutic trial, revealed increased ara-C-derived radioactivity in combination with THU (1000 mg/kg) only in the intestines. All other tissues, including tumor and blood, showed no overall in-

TABLE 3.—Deaminase and kinase values in kidneys and E0771 tumors after a single administration of THU

THU dose (mg/kg)	Route of administration	Time of evaluation after injection	Ara-C deaminase* (% treated/control)		dCR kinase* (% treated/control)
			Tumor	Kidney	Tumor
10	ip	30 mins	−27†	+17†	−12†
100	ip	30 mins	−72	−75	−10
1000	ip	30 mins	−73	−100	+1
100	sc	30 mins	−17	−36	−9
1000	sc	30 mins	−26	−74	+10
10	ip	2 hrs	+3	+8	−1
100	ip	2 hrs	−11	−33	−12
1000	ip	2 hrs	−65	−81	−4
100	sc	2 hrs	−36	−50	−3
1000	sc	2 hrs	−41	−65	−19

*8 tumors and 8 kidneys were pooled and homogenized for enzyme assays. The values indicated are the means of 3 assays each. Control values for ara-C deaminase: tumor, 165 nmols/mg/hr, and kidney, 879 nmols/mg/hr; control values for dCR kinase: tumor, 3.5 nmols/mg/hr.

†− = inhibition; + = activation of enzyme activity.

crease, but rather a decrease in ara-C-derived radioactivity within 1–48 hours after the simultaneous injection of the two substances (fig 1). A rapid exponential decrease of radioactivity was found in all tissues investigated with the exception of the small intestine and brain.

THU-derived radioactivity showed a relatively high initial F value only in the kidney, followed by considerably lower levels in the blood, liver, small intestine, tumor, and spleen, and very low values in the brain tissue. A rapid exponential decrease of radioactivity was found in all tissues tested, except the small intestine, where a peak was reached at 8 hours with a subsequent rapid decrease of the radioactivity The initial half-life of THU-derived radioactivity in the blood was slightly lower (about 1 hour) than that of ara-C-derived radioactivity.

The effect of THU upon the ara-C plasma levels is demonstrated in figure 2. Whereas THU at 1000 mg/kg given ip in combination with ara-C at 15 mg/kg to S180J ascites-bearing BDF_1 mice produced a slight overall decrease of total ara-C equivalents compared to the levels observed after ara-C alone, analysis for unchanged ara-C revealed overall somewhat higher ara-C plasma levels under the influence of the combined treatment. This change also resulted in about a threefold increase in the half-life of ara-C in the combination treatment compared with the half-life obtained when ara-C was administered alone (about 30 minutes).

The direct effect of THU upon the intracellular CR deaminase was evaluated in vivo in E0771, the tumor with the overall highest CR-deaminase levels (table 3). Eight to 10 days after sc implantation of this tumor into BDF_1 mice, enzyme levels were evaluated at 30 minutes and 2 hours after a single ip or sc administration of 10, 100, or 1000 mg/kg of THU. A striking dose-dependent inhibitory effect on both host kidney and tumor deaminase (ara-C as substrate) was observed. The effect was most distinct with 1000 mg/kg of THU when, at 30 minutes after ip injection, 100% deaminase inhibition was obtained in the kidney and 73% in the tumor. Two hours after the ip injection the deaminase inhibition was still significant in both tissues, although to a slightly reduced level. After sc injection of 100 or 1000 mg/kg of THU, the deaminase inhibitory effect in the tumor was greater 2 hours after the injection than 30 minutes after the administration. In the host kidney, deaminase inhibition was higher 2 hours rather than 30 minutes after 100 mg/kg of THU sc, but higher 30 minutes rather than 2 hours after 1000 mg/kg of THU sc. The effect of THU upon the dCR-kinase levels in these tissues is significant only in the tumor tissue in the form of a 19% reduction 2 hours after the sc administration of 1000 mg/kg of THU. The discrepancy between the kinase values of this experiment and the ones reported in table 1 is probably due to different ages of the tumors. Differences in the kinase values in different cell lines of the same tumor were also reported by Furner and Mellett (47).

The most striking difference in the effect of THU was found in the intracellular distribution pattern

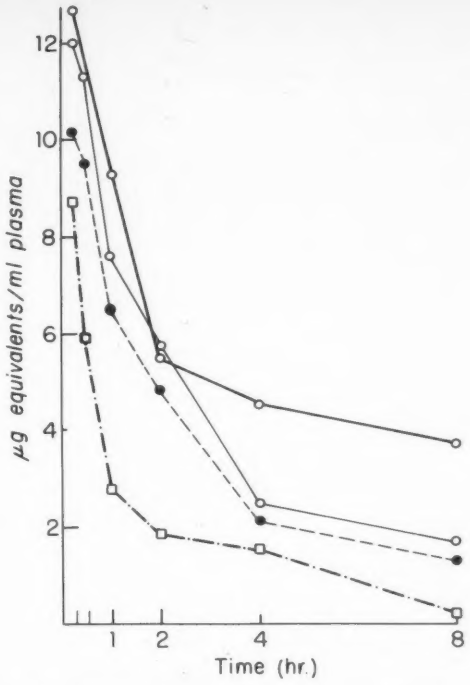


FIGURE 2.—Plasma levels in S180J ascites-bearing mice after an ip injection of 15 mg/kg of [^{3}H]-ara-C alone or in combination with 1000 mg/kg of THU. Total ara-C equivalents (○——○); ara-C (□-·-□) after [^{3}H]-ara-C was given alone. Total ara-C equivalents (○——○); ara-C (●----●) after [^{3}H]-ara-C was given in combination with THU. Abscissa: time tissues removed after injection; ordinate: μg ara-C equivalents or ara-C per ml of plasma. For methods *see* text.

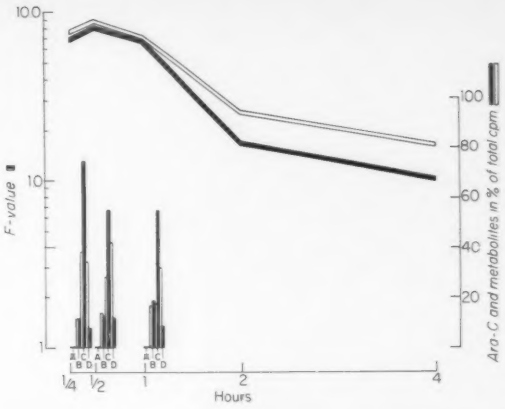


FIGURE 3.—Cellular distribution and metabolism of ara-C in S180J solid tumor after an ip injection of 15 mg/kg of [^{3}H]-ara-C alone (▭) or in combination with 1000 mg/kg of THU(▬). Vertical bars indicate A: ara-C-derived 5′-di- and tri-phosphates; B: ara-C-derived 5′-mono-phosphate; C: ara-C; D: ara-U. Abscissa: time after injection; ordinate: (left) modified F value; (right) percentage of ara-C, ara-U, and metabolites at different times after injection. ▯ = experiment with ara-C alone; ▮ = experiment with ara-C + THU. Limit of detection for ara-C, ara-U, and their 5′-mono-, di-, or tri-phosphates is 0.064 nmol.

of ara-C-derived radioactivity in the solid compared to the ascites form of S180J and was expressed in the overall reduction of the F values in the homogenate of solid tumors (fig 3) compared to the protracted increase of the F values in the homogenate of the ascites tumor (fig 4).

The analysis by paper chromatography of acid-soluble extracts from homogenates of these cells reveals the prevalence of 5′-di- and tri-phosphates of ara-C in the ascites cells at 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, and 8 hours after the ip injection of the drug (fig 4), and the striking absence of these di- and tri-phosphates in the solid form at 15 minutes, 30 minutes, and 1 hour after the ip injection (fig 3). In turn, in the ascites form, the nucleosides and 5′-mono-phosphates are low, whereas in the solid form, ara-C and ara-U are predominant and the 5′-mono-phosphates are the only ara-C-derived phosphates present in the solid tumor form.

The influence of THU upon the quantity of di- and tri-phosphates in the ascites cells is visible only ½ and 1 hour after the injection of the drug and is expressed in the form of a relative increase of these nucleotides. In the solid form, a striking increase of the ara-C over the ara-U was found 15 minutes, 30 minutes, and 1 hour after the ip injection of ara-C under the influence of THU. High-pressure liquid chromatography of the same PCA extracts confirmed these findings and revealed that the reported 5′-di- and tri-phosphates (fig 4) were composed of about equal amounts of ara-CDP and ara-CTP. 5′-Mono-, di-, and tri-phosphates of ara-U were detected in only minute amounts.

DISCUSSION

For the objective of this study with respect to deaminase activity, the most pertinent tumor should have been E0771. However, neither this nor the other solid tumors tested exhibited any significant response to ara-C alone or to the combination of ara-C + THU without also resulting in toxicity in the form of severe weight loss or death. Only the ascites form of S180J showed a therapeutic response to ara-C alone or in combination with THU. These ascites cells also revealed the highest amount of

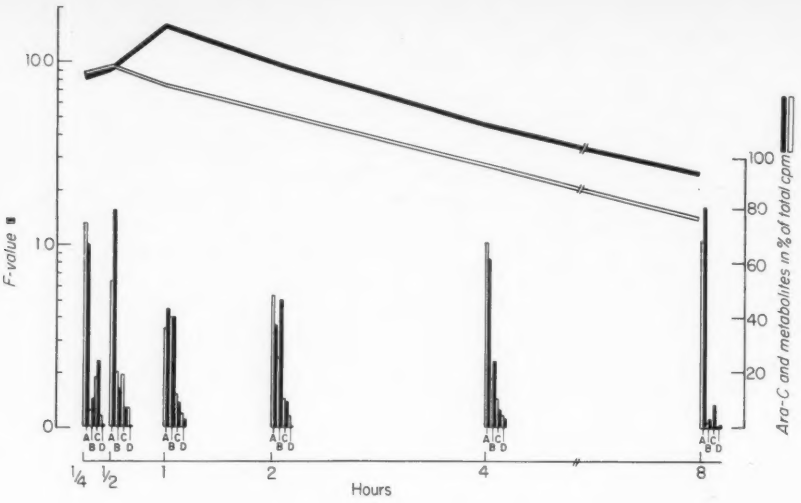


FIGURE 4.—Cellular distribution and metabolism in S180J ascites tumor after an ip injection of 15 mg/kg of [^{3}H]-ara-C alone or in combination with 1000 mg/kg of THU. For symbols *see* fig 3.

dCR-kinase activity of all the neoplasms studied except the P815 mouse neoplasm.

The poor response of the solid tumors can partly be explained by the effect THU had on the distribution of [^{3}H]-ara-C in T241-bearing BDF_1 mice. A significant reduction in the accumulation of ara-C-derived radioactivity was found in tumor and host tissues under the influence of simultaneously administered THU at 1000 mg/kg and was most prominent at 8 hours in the liver (to 40%) and the tumor (to 45%), less prominent in the kidneys, brain, spleen, and blood, and no reduction at all occurred in the small intestines. Furthermore, in all of the tissues except the small intestines, THU-derived radioactivity was cleared faster than ara-C-derived radioactivity.

CR-deaminase assays performed on 8–10-day-old E0771 tumors removed from their BDF_1 hosts ½ or 2 hours after a single ip injection of 1000 mg/kg of THU clearly demonstrated significant inhibition of this enzyme in the tumor as well as in the host kidneys. A slight inhibitory effect of THU upon the dCR kinase indicates that the inability of THU to increase the chemotherapeutic activity of ara-C in solid tumors is probably due to effects other than those observed on the intracellular levels of these two key enzymes.

A direct interference, such as complex formation (intracellular or extracellular), of ara-C and THU is unlikely since THU has no effect in vitro upon the ultraviolet spectrum of ara-C.

An ideal system for further studies was found in the S180J tumor system. The difference in the response of solid and ascites forms of S180J, both inoculated by the same ascites cells, can be explained by the intracellular distribution of ara-C and its metabolites in the two tumor forms. While in the solid form, a decrease of ara-C-derived radioactivity was observed under the influence of simultaneously administered THU, the use of THU produced a specific and protracted accumulation of ara-C-derived radioactivity in the ascites form. More ara-C-derived radioactivity was found at any time over the 8-hour observation period in the combination experiment than when ara-C was given alone. Intracellular analysis, both by paper chromatography and high-pressure liquid chromatography, revealed no ara-C 5′-di- and tri-phosphates in the solid tumor extracts, but a predominance of ara-C in the combination experiment and a predominance of ara-U when [^{3}H]-ara-C was injected alone. The predominant radioactive material in the ascites extracts was found to be ara-CDP and ara-CTP with only small amounts of ara-C and ara-U. Furthermore, the influence of THU upon the ara-CDP and ara-CTP levels in the ascites form was reflected in a relative increase of ara-C di- and tri-phosphates at least within ½ and 1 hour after the injection. The lack of response to ara-C in the solid tumors of S180J, E0771, T241, and LL was in contrast to P815; there, as reported earlier, both ascites and solid tumors responded equally well to the same dosage of ara-C (12).

The influence of THU upon the ara-C blood levels of the BDF_1 host is only a moderate one and might explain the moderate effect or lack of effect of THU + ara-C in chemotherapeutic trials in experimental test systems lacking an intracellular CR deaminase, such as L1210 (38) or P815 (Kreis W, unpublished results) mouse neoplasms. Thus, THU will probably benefit the treatment with ara-C in neoplasms which have sufficient kinase activity, and a beneficial effect will more likely be due to intracellular events rather than to elevated ara-C blood levels; it is likely that leukemias will respond better to this combination than solid tumors. Our observations with the ascites form of S180J concur in principle with the observations of Chou et al (49). They reported an increased production of ara-CTP from ara-C under the influence of THU in tissue cultures of human AML and acute myelomonocytic leukemia. Normal cells or cells from other leukemias showed less or negligible THU effects (49).

REFERENCES

1. HO DHW. Distribution of kinase and deaminase of 1-β-D-arabinofuranosylcytosine in tissues of man and mouse. Cancer Res 33:2816–2820, 1973.
2. CAMIENER GW, and SMITH CG. Studies on the enzymatic deamination of cytosine arabinoside. I. Enzyme distribution and species specificity. Biochem Pharmacol 14:1405–1406, 1965.
3. STEUART CD, and BURKE PJ. Cytidine deaminase and the development of resistance to arabinosylcytosine. Nature (New Biol) 233:109–110, 1971.
4. HO DHW, and FREI E, III. Clinical pharmacology of 1-β-D-arabinofuranosylcytosine (ara-C). Clin Pharmacol Ther 12:944–953, 1971.
5. ABELL CW, and MARCHAND NW. Cytidine deaminase in human lymphocytes. Nature (New Biol) 244:217–219, 1973.
6. COLEMAN CN, JOHNS DG, and CHABNER BA. Studies on mechanisms of resistance to cytosine arabinoside: problems in the determination of related enzyme activities in leukemic cells. Ann NY Acad Sci 255:247–251, 1975.
7. HART JS, HO DHW, GEORGE SL, ET AL. Cytokinetic and molecular pharmacology studies of arabinosylcytosine in metastatic melanoma. Cancer Res 32:2711–2716, 1972.
8. BARRANCO SC, HO DHW, DREWINKO B, ET AL. Differential sensitivities of human melanoma cells grown in vitro to arabinosylcytosine. Cancer Res 32:2733–2736, 1972.
9. FREI E, HO DHW, BODEY GP, ET AL. Pharmacologic and cytokinetic studies of arabinosylcytosine. *In* Verifying Concepts of Leukemia (Dutcher RM, and Chieco-Bianchi L, eds). Bibl Haematol 39:1085–1097, 1973.
10. TATTERSALL MHN, GANESHAGURU K, and HOFFBRAND AV. Mechanisms of resistance of human acute leukemia cells to cytosine arabinoside. Br J Haematol 27:39–46, 1974.
11. HIRSCHMANN WD, CHRISKE HW, KOVACS EL, ET AL. Biochemische und pharmakokinetische Aspekte zur klinischen Anwendung von cytosinarabinosid bei akuten leukämien. Blut 28:237–238, 1974.
12. UCHIDA K, and KREIS W. Studies on drug resistance—I. Distribution of 1-β-D-arabinofuranosylcytosine, cytidine and deoxycytidine in mice bearing ara-C-sensitive and -resistant P815 neoplasms. Biochem Pharmacol 18:1115–1128, 1969.
13. CHU MY, and FISCHER GA. Comparative studies of leukemic cells sensitive and resistant to cytosine arabinoside. Biochem Pharmacol 14:333–341, 1965.
14. KESSEL D, HALL TC, and WODINSKY I. Transport and phosphorylation as factors in the antitumor action of cytosine arabinoside. Science 156:1240–1241, 1967.
15. SCHRECKER AW. Metabolism of 1-β-D-arabinofuranosylcytosine in leukemia L1210: nucleoside and nucleotide kinases in cell-free extracts. Cancer Res 30:632–641, 1970.
16. KESSEL D, HALL TC, and ROSENTHAL D. Uptake and phosphorylation of cytosine arabinoside by normal and leukemic human blood cells in vitro. Cancer Res 29:459–463, 1969.
17. MOMPARLER RL, CHU MY, and FISCHER GA. Studies on a new mechanism of resistance to L5178Y murine leukemic cells to cytosine arabinoside. Biochim Biophys Acta 161:481–493, 1968.
18. BACH MK. Biochemical and genetic studies of a mutant strain of mouse leukemia L1210 resistant to 1-β-D-arabinofuranosylcytosine (cytarabine) hydrochloride. Cancer Res 29:1036–1044, 1969.
19. DURHAM JP, and IVES DH. Deoxycytidine kinase. I. Distribution in normal and neoplastic tissue and interrelationships of deoxycytidine and 1-β-D-arabinofuranosylcytosine phosphorylation. Mol Pharmacol 5:358–375, 1969.
20. DOLLINGER MR, BURCHENAL JH, KREIS W, ET AL. Analogs of 1-β-D-arabinofuranosylcytosine. Studies on mechanism of action of Burkitt's cell culture and mouse leukemia and in vitro deamination studies. Biochem Pharmacol 16:689–706, 1967.
21. CAMIENER GW. Studies of the enzymatic deamination of cytosine arabinoside. III. Substrate requirements and inhibition of the deaminase of human liver. Biochem Pharmacol 16:1691–1702, 1967.
22. CAMIENER GW, and TAO RV. Studies of the enzymatic deamination of ara-cytidine. IV. Inhibition by acridine analogue and organic solvents. Biochem Pharmacol 17:1411–1423, 1968.
23. CAMIENER GW. Studies of the enzymatic deamination of ara-cytidine. V. Inhibition in vitro and in vivo by tetrahydrouridine and other reduced pyrimidine nucleosides. Biochem Pharmacol 17:1981–1991, 1968.
24. NEIL GL, BUSKIRK HH, MOXLEY TE, ET AL. Biochemical and pharmacological studies with 1-β-D-arabinofuranosyl cytosine 5'-adamantoate (NSC-117614), a depot form of cytarabine. Biochem Pharmacol 20:3295–3308, 1971.
25. DARZYNKIEWICZ E, KUSMIEREK JJ, and SHUGAR D. *O'*-methyl-derivatives of arabinosylcytosine. Biochem Biophys Res Commun 46:1734–1741, 1972.
26. KANAI T, and ICHINO M. Pyrimidine nucleosides. 5. Synthesis of carcinostatic halogeno cyclonucleosides. J Med Chem 15:1218–1221, 1972.
27. HOSHI A, KANZAWA G, KURETANI K, ET AL. Antitumor activity of cyclocytidine derivatives. Gann 64:519–522, 1973.
28. FOX JJ, FALCO EA, WEMPEN I, ET AL. Oral and parenteral activity of 2,2'-anhydro-1-β-D-arabinofuranosyl-5-fluorocytosine against both intraperitoneally and intracerebrally inoculated mouse leukemia. Cancer Res 32:2269–2272, 1972.
29. HANZE AR. Nucleic acids. IV. The catalytic reduction of pyrimidine nucleosides (human liver deaminase inhibitors). J Am Chem Soc 89:6720–6725, 1967.
30. HO DHW. Inhibition of 1-β-D-arabinofuranosyl cytosine phosphorylation in human liver by tetrahydrouridine. Biochem Pharmacol 21:2936–2940, 1972.
31. MULLIGAN LT, and MELLETT LB. Inhibition of arabinosylcytosine deamination by tetrahydrouridine in the monkey. Pharmacologist 12:221, 1970.
32. EL DAREER SM, MELLETT LB, WHITE V, ET AL. Inhibition of deamination of ^{3}H-arabinosylcytosine (ara-C) by tetrahy-

drouridine (H_4U) in BDF_1 mice. Pharmacologist 16:210, 1974.
33. MULLIGAN LT, JR, and MELLETT LB. Comparative metabolism of cytosine arabinoside and inhibition of deamination by tetrahydrouridine. Pharmacologist 10:167, 1968.
34. GOLDENTHAL EI, COOKSON KM, GEIL RG, ET AL. Preclinical toxicologic evaluation of tetrahydrouridine (NSC-112907) in beagle dogs and rhesus monkeys. Cancer Chemother Rep Part 3, vol 5(1):15–16, 1974.
35. FURNER RL, MELLETT LB, and HERREN TC. Phosphorylation of 1-β-D-arabinofuranosylcytosine (ara-C) in the presence of tetrahydrouridine (H_4U): kinetics of ara-C metabolism by solid tumors in vitro. Pharmacologist 16:210, 1974.
36. CHABNER BA, JOHNS DG, COLEMAN CN, ET AL. Purification and properties of cytidine deaminase from normal and leukemic granulocytes. J Clin Invest 53:922–931, 1974.
37. WENTWORTH DF, and WOLFENDEN R. On the interaction of 3,4,5,6-tetrahydrouridine with human liver cytidine deaminase. Biochemistry 14:5099–5105, 1975.
38. NEIL GL, MOXLEY TE, and MANAK RC. Enhancement by tetrahydrouridine of 1-β-D-arabinofuranosylcytosine (cytarabine) oral activity in L1210 leukemic mice. Cancer Res 30:2166–2172, 1970.
39. COOPER GH, and GREER S. The effect of inhibition of cytidine deaminase by tetrahydrouridine on the utilization of deoxycytidine and 5-bromodeoxycytidine for deoxyribonucleic acid synthesis. Mol Pharmacol 9:698–703, 1973.
40. KREBS HA. Body size and tissue respiration. Biochim Biophys Acta 4:249–296, 1950.
41. ROTH JS, LEAGUE M, and KOTHS M. The effect of whole-body gamma irradiation on the deoxycytidylate, deoxyadenosine and deoxyguanosine deaminase activity of rat spleen and thymus. Radiat Res 22:722–729, 1964.
42. KREIS W, GREENSPAN A, WOODCOCK T, ET AL. Separation of 5'-mono-, di- and triphosphates of 1-β-D-arabinofuranosyluracil by thin-layer and high pressure liquid chromatography. J Chromatogr Sci 14:331–333, 1976.
43. KREIS W, GORDON C, GIZONI C, ET AL. Extraction and analytic procedures for cytosine arabinoside and 1-β-D-arabinofuranosyluracil and their 5'-mono-, di- and tri-phosphates. Cancer Treat Rep 61:643–646, 1977.
44. CARPENTER DC. Paper chromatography of nucleic acid derivatives. Anal Chem 24:1203–1204, 1952.
45. DOLLINGER MR, BURCHENAL JH, KREIS W, ET AL. Analogs of 1-β-D-arabinofuranosylcytosine studies on mechanisms of action in Burkitt's cell culture and mouse leukemia, and in vitro deamination studies. Biochem Pharmacol 16:689–706, 1967.
46. LOWRY OH, ROSEBROUGH NJ, FARR AL, ET AL. Protein measurements with the Folin phenol reagent. J Biol Chem 193:265–275, 1951.
47. FURNER RL, and MELLETT LB. Kinase and deaminase activity in a variety of subcutaneous mouse tumors. Cancer Res 35:1799–1803, 1975.
48. TARNOWSKI GS, SCHMID FA, CAPPUCCINO JG, ET AL. Chemotherapy studies in an animal tumor spectrum. II. Sensitivity of tumors to fourteen antitumor chemicals. Cancer Res 26(part 2):181–206, 1966.
49. CHOU TC, ARLIN Z, CLARKSON B, ET AL. Effect of tetrahydrouridine (THU) on metabolism of cytosine arabinoside (ara-C) in human leukemic cells. Proc Am Assoc Cancer Res and ASCO 17:142, 1976.

Phase II Trial With Bleomycin, CCNU, and Streptozotocin in Patients With Metastatic Cancer of the Breast [1,2]

Brief Reports and Preliminary Communications

P. R. Band,* G. P. Canellos, M. Sears, L. Israel, and S. J. Pocock [3]

In 1971, the Breast Cancer Committee of the Eastern Cooperative Oncology Group developed a phase II protocol designed to screen new drugs for antitumor activity in patients with breast cancer, and to obtain further information on the toxic effects of these agents. This paper reports the results obtained with the three drugs initially studied: bleomycin, CCNU, and streptozotocin. Bleomycin was chosen because of its activity in a variety of tumor types (1) and for its lack of hematologic toxicity. CCNU, a synthetic nitrosourea derivative, was selected because of its reported activity in some human solid tumors (2) and because it can be administered orally, an advantage it has over BCNU. Streptozotocin, a naturally occurring nitrosourea antibiotic, was chosen because of its lack of marrow toxicity (3).

PATIENTS AND METHODS

Sixty-four of 78 patients who were entered in the study were evaluable for tumor response and for toxicity. Eight patients were initially ineligible because of major protocol violations; six were unevaluable because of inadequate tumor measurements. Each patient received treatment which was assigned on a random basis. The three drugs used in this study were: bleomycin (15 mg/m²) given im once weekly; CCNU (130 mg/m²) given orally as a single dose and repeated every 6 weeks (this dose proved to be too toxic and was subsequently reduced to 100 mg/m²); and streptozotocin (1.25 g/m²) given iv once weekly. Because of poor gastrointestinal tolerance, this dosage of streptozotocin was reduced early in the study to 0.5 g/m²/week.

TUMOR RESPONSE

Patient characteristics, response to treatment, and toxicity are indicated in table 1. The distribution of patient ages, prior therapy, performance status, and disease dominance was similar in each treatment group. Seventy-three percent of the patients had received prior chemotherapy and 58% had received prior hormonal therapy. Only 55% of the patients were initially ambulatory. Seventy-three percent of the patients had visceral disease; bone and soft tissue involvement were also present in 45% and 77% of the patients respectively. The median times on study for patients receiving bleomycin, CCNU, and streptozotocin were 53, 63, and 55 days respectively. Only four partial responses were observed.

TOXICITY (TABLE 2)

Bleomycin caused no significant hematologic or gastrointestinal toxicity. Skin toxic effects occurred in six patients and were considered severe in two. Moderate chills and fever developed in five patients and severe pulmonary fibrosis developed in one. Hematologic toxic effects were common in patients receiving CCNU, with severe and life-threatening

[1]Received July 1, 1976; revised Apr 20, 1977; accepted May 25, 1977.

[2]Bleomycin: NSC-125066; CAS reg. No. 11056-06-7; [2,4'-bithiazole]-4-carboxylic acid, 2'-(2-aminoethyl)-, monohydrate.

CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-.

Streptozotocin: NSC-85998.

[3]This study was performed by the Eastern Cooperative Oncology Group (*Paul P. Carbone*, chairman; CM-33745), and was supported by Public Health Service grants from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare. The following institutions participated in the study: University of Alberta, Canada (*P. R. Band;* CA-13314); NCI, Bethesda, Md (*G. P. Canellos*); University of Rochester, NY (*M. Sears;* CA-11083); Centre Hospitalier Universitaire Lariboisière, Paris, France (*L. Israel*); State University of New York, Buffalo (*S. J. Pocock;* CA-12721). Other participating institutions were: Albany Medical College (CA-06594); Albert Einstein College of Medicine (CA-16958); American Oncologic Hospital; Boston University Medical Center (CA-11109); Case Western Reserve University (CA-14548); Chicago Medical School at Mt. Sinai Hospital (CA-14144); Georgetown University School of Medicine (CA-02824); Hahnemann Medical College (CA-13611); Jackson Memorial Hospital (CA-02822); Jefferson Medical College (CA-14215); Maimonides Medical Center (CA-05588); Medical College of Virginia (CA-10572); Mt. Sinai Medical Center; State University of New York; Tufts University School of Medicine (CA-07190); University of Iowa; University of Ottawa and Ontario Cancer Foundation; University of Pennsylvania; and University of Pretoria.

Reprint requests to: P. R. Band, MD, Institut du Cancer de Montréal, Centre Hospitalier Notre-Dame, 1560 est, Sherbrooke, Montréal, Canada, H2L 4M1.

TABLE 1.—Patient characteristics and response to treatments

Characteristics	Treatment			Totals	%
	Bleomycin	CCNU	Strepto-zotocin		
No. of patients	24	20	20	64	100
Age (yrs)					
< 50	6	3	2	11	17
50–59	10	6	11	27	42
60–69	8	8	6	22	34
≥ 70	0	3	1	4	6
Prior—					
Radiotherapy	19	17	16	52	81
Hormonal therapy	13	9	15	37	58
Chemotherapy	19	15	13	47	73
Initial performance status					
Ambulatory	14	10	11	35	55
Nonambulatory	10	10	9	29	45
Disease dominance					
Soft tissue	4	2	2	8	13
Osseous	4	1	4	9	14
Visceral	16	17	14	47	73
Response					
Complete	0	0	0	0	—
Partial	2	2	0	4	6
Median survival (wks)	25	13	9	—	—
Hematologic toxicity					
Mild	1	3*	6†	10	16
Moderate	0	7‡	1	8	13
Severe	0	5	1	6	9
Life threatening	0	5	0	5	8
Gastrointestinal toxicity					
Mild	5	7*	2	14	22
Moderate	2	4	6†	12	19
Severe	0	1*	8	9	14
Life threatening	0	0	0	0	—

*1 patient treated at a dose of 100 mg/m².
†1 patient treated at a dose of 1.25 g/m².
‡2 patients treated at a dose of 100 mg/m².

TABLE 2.—Classification of severity of hematologic and gastrointestinal toxicity

Toxic effects	Degree			
	Mild	Moderate	Severe	Life threatening
Hematologic				
Wbc count (cells/mm³)	3000–3999	2000–2999	1000–1999	< 1000
Platelet count (platelets/mm³)	75,000–99,999	50,000–74,999	30,000–49,999	< 30,000
Gastrointestinal	Nausea	Intermittent vomiting controllable by antiemetics	Continuous vomiting, partly controllable by antiemetics	Intractable vomiting

thrombocytopenia or leukopenia occurring in ten. Most patients experienced some gastrointestinal side effects that were usually mild or moderate in degree. For patients receiving streptozotocin, the major complication was gastrointestinal in nature, with moderate and severe vomiting occurring in 14 patients. Hematologic toxicity was generally mild. Mild proteinuria (< 1 g/24 hours) occurred in two patients.

COMMENT

None of the three drug treatments induced a significant incidence of tumor regression. The two partial responses observed with CCNU were accompanied by severe hematologic toxicity. Bleomycin induced partial remission in two patients with visceral disease without producing significant hematologic or gastrointestinal toxicity. Hematologic toxicity due to CCNU and gastrointestinal side effects due to streptozotocin were severe in the majority of patients, while treatment with bleomycin was generally well tolerated.

The disappointing results observed in this phase II study may be related to several factors: most patients had visceral involvement and were in the late stages of their disease; the majority of patients had received extensive prior therapy with 73% having failed or relapsed on previous chemotherapy. However, the incidence of objective responses observed for CCNU in this study does not differ from that noted by Ahmann et al (4) in patients with disseminated breast cancer not previously exposed to chemotherapeutic drugs.

REFERENCES

1. Clinical Screening Cooperative Group of the European Organization for Research on the Treatment of Cancer. Study of the clinical efficiency of bleomycin in human cancer. Br Med J 2(5710):643–645, 1970.
2. Hansen HH, Selawry OS, Muggia FM, et al. Clinical studies with 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (NSC 79037). Cancer Res 31:223–227, 1971.
3. Moertel CG, Reitemeier RJ, Schutt AJ, et al. Phase II study of streptozotocin (NSC-85998) in the treatment of advanced gastrointestinal cancer. Cancer Chemother Rep 55:303–307, 1971.
4. Ahmann DL, Bisel HF, and Hahn RG. Difficulties designing clinical trials as exemplified by a phase 2 drug evaluation of 5[3,3-bis(2-chloroethyl)-1-triazenol]-imidazole-4-carboxamide and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea in patients with disseminated breast cancer. Cancer Res 33:1707–1710, 1973.

Treatment of Advanced Ovarian Carcinoma With High-Dose Cyclophosphamide After Failure on Melphalan[1,2]

Brief Reports and Preliminary Communications

Leo B. Twiggs* and C. Paul Morrow[3]

Alkylating agents have been the most active cytotoxic drugs in advanced ovarian carcinoma, with an objective response rate of 50% commonly reported (1–3). Despite their activity as first-line drugs, they seldom have been used in alkylating-drug failures because of presumed cross resistance. High-dose cyclophosphamide (CTX) (120 mg/kg) has been reported by Buckner et al (4) to yield objective responses in eight of nine previously untreated patients suggesting a level of activity superior to other regimens. In this study high-dose CTX was tested for activity against ovarian carcinoma resistant to melphalan.

METHODS

Thirteen patients with advanced ovarian carcinoma (equivalent to FIGO stage III or IV) resistant to melphalan are included in this study. Three patients had also received abdominal irradiation. The histopathology was serous in nine patients; the others were mixed serous and mucinous, clear cell, mucinous, endometrioid, and undifferentiated in one patient each. Ten patients originally had surgical stage III disease, two had stage IV, and one had stage IB.

Each patient was treated with CTX at a dose of 1.0 g/m^2 iv repeated at 3-week intervals or when recovery from toxicity was complete. The drug dose was reduced by 25% for patients with (*a*) previous abdominal irradiation, (*b*) more than three previous courses of chemotherapy, or (*c*) poor physical condition (Karnofsky performance score < 50%). Individual patient characteristics are summarized in table 1 including primary chemotherapy, response to that therapy, response to CTX therapy, and total duration of therapy.

Patients were considered evaluable if they received at least two courses of drug. Response is defined as follows: *complete response*—disappearance of all measurable tumor for ≥ 3 months; *partial response*—a 50% decrease in palpable tumor size or control of serous effusions for ≥ 3 months; *stable disease*—< 50% reduction in tumor; and *progression*—new lesions or > 50% increase in palpable tumor. Toxicity criteria are presented in table 2.

RESULTS

Ten of 13 patients receiving high-dose CTX were evaluable. Only two objective responses occurred. One responder had a 50% reduction in a palpable abdominal mass sustained for six courses of CTX. The other had control of ascites lasting 18 months and received 24 courses of CTX therapy. Both of these patients had a partial response to melphalan prior to treatment with CTX.

In 13 patients 57 courses of CTX were administered (range, one to 24 courses). Eight patients received more than three courses of the drug. In the six nonresponders the drug treatment was continued because of stable or slowly progressive disease. Three patients were unevaluable because they received only one course of drug.

TOXICITY

Among the 57 courses of pulse CTX there were five episodes of severe or life-threatening hematologic toxicity occurring in four patients. One patient had two episodes of life-threatening leukopenia with nadir wbc counts of 900/mm^3; another patient had a nadir wbc count < 1500/mm^3 and thrombocytopenia with a platelet count of 45,000/mm^3 after her only course of drug therapy. Two patients had single episodes of life-threatening leukopenia complicated by sepsis. One patient with widespread disease and a small bowel obstruction died with intra-abdominal abscesses and bronchopneumonia 6 days after treatment. Most patients had gastrointestinal toxicity in

[1]Received Dec 13, 1976; revised Mar 14, 1977; accepted Apr 20, 1977.

[2]Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

Melphalan: NSC-8806; CAS reg. No. 3223-07-2; alanine, 3-[*p*-[bis(2-chloroethyl)amino]phenyl]-, monohydrochloride, L-.

[3]Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Los Angeles (LA) County, University of Southern California (USC) Medical Center.

**Reprint requests to:* Leo B. Twiggs, MD, Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, LA County, USC Medical Center, 1240 N Mission Rd, Los Angeles, Calif 90033.

the form of nausea and vomiting but in none was it severe.

DISCUSSION

The efficacy of CTX as initial chemotherapy in advanced ovarian carcinoma has been well documented (1,3). Its apparently greater response rate when administered in a high-dose bolus has prompted its application to patients who have failed on melphalan (4). Piver et al (5) reported only one partial response lasting 1 month among seven patients with ovarian cancer refractory to alkylating-drug therapy. Three of his patients had received low-dose CTX in combination with actinomycin D and 5-FU or methotrexate prior to receiving high-dose CTX. Although none of our patients had received prior CTX, the response rate was comparable (two of ten patients, 20%) with no complete responses. Toxicity from this drug regimen was considerable. In our study four of 13 patients (30%) had severe or life-threatening leukopenia with two of four (50%) complicated by septicemia. In Piver's group six of eight patients (75%) had severe leuko-

TABLE 1.—Patient characteristics

Patient No.	Primary chemotherapy, No. of cycles, duration in mos	Response to primary chemotherapy	Response to CTX chemotherapy	Total duration of therapy (mos)
1	Melphalan, 2,3	Progression	Progression	5
2*	Melphalan, 5,6	Stable	Unevaluable	12
3	Melphalan, 7,8	Stable	Unevaluable	10
4	Melphalan, 7,8	Partial	Unevaluable	13
5	Melphalan, 8,10 Melphalan, hexamethylmelamine, 1	Partial	Partial	14
6	Melphalan, 9,12	Partial	Progression	16
7†	Melphalan, 2,3	Progression	Progression	6
8*	Melphalan, actinomycin D, 5-FU, 7,10	Partial	Partial	26
9	Melphalan, 5-FU, 12,12	Complete	Progression	18
10	Melphalan, 4,6	Progression	Progression	9
11	Melphalan, 3,3	Progression	Progression	5
12	Melphalan, hexamethylmelamine, 5,7	Progression	Progression	9
13*	Melphalan, 6,12	Partial	Progression	16

*Also received abdominal irradiation.
†Originally stage IB.

TABLE 2.—Adverse effects criteria

	Effect			
	Mild	Moderate	Severe	Life threatening
Wbc (cells/mm^3)	3000–3999	2000–2999	1000–1999	< 1000
Granulocytes (cells/mm^3)	1000–1499	500–599	250–499	< 250
Platelets (cells/mm^3)	100,000–149,000	50,000–99,999	25,000–49,999	< 25,000
Nausea and vomiting	Nausea only	Vomiting controlled by antiemetics	Frequent vomiting despite antiemetics	—

penia with five manifesting septicemia. It is clear that the risks of high-dose bolus CTX therapy for ovarian carcinoma resistant to melphalan outweigh the potential benefits.

REFERENCES

1. BECK RE, and BOYES DA. Treatment of 126 cases of advanced ovarian carcinoma with cyclophosphamide. Can Med Assoc J 98:539–541, 1968.
2. SMITH JP, and RUTLEDGE F. Chemotherapy in the treatment of cancer of the ovary. Am J Obstet Gynecol 107:691–703, 1970.
3. YOUNG RC, CANELLOS GP, CHABNER BA, ET AL. Chemotherapy of advanced ovarian carcinoma: a prospective randomized comparison of phenylalanine mustard and high dose cyclophosphamide. Gynecol Oncol 2:489–497, 1974.
4. BUCKNER CD, BRIGGS R, CLIFT RA, ET AL. Intermittent high-dose cyclophosphamide (NSC-26271) treatment of stage III ovarian carcinoma. Cancer Chemother Rep 58:697–703, 1974.
5. PIVER MS, BARLOW JJ, and CHUNG WS. High-dose cyclophosphamide (NSC-26271) for recurrent or progressive ovarian adenocarcinoma. Cancer Chemother Rep 59:1157–1158, 1975.

Phase II Trial of Asaley in Children With Late-Stage Acute Lymphocytic Leukemia [1,2,3,4]

Brief Reports and Preliminary Communications

Charles L. Sexauer,[5,*] Samuel Morgan,[6] Jan van Eys,[7] and Diane M. Komp [8]

Larionov has demonstrated that the addition of amino acids to the acetylated form of the alkylating agent melphalan altered the toxicity and the antineoplastic activity of the parent compound. The most promising of these derivatives, asaley, is the acetyl-L-leucine derivative of melphalan (1).[9] Animal tumor data demonstrated asaley to be superior to melphalan in Sarcoma 180 and Sarcoma 289 (1). In Russia, response rates of 43% and 50% were achieved in a clinical trial in Hodgkin's disease and reticulum cell sarcoma (2). Because of these responses, a phase II trial was initiated by the Pediatric Division of the Southwest Oncology Group to determine if asaley might be active in acute lymphocytic leukemia (ALL) in childhood.

MATERIAL AND METHODS

Patients with ALL were eligible for study if they were: (*a*) < 18 years of age at the time of diagnosis and < 21 years of age at the time of this relapse; (*b*) in bone marrow relapse (M-3 marrow status); (*c*) resistant to drugs of proven value in ALL; (*d*) had not received chemotherapeutic treatment for at least 1 week with the exception of prednisone; and (*e*) were not in a toxic condition from prior therapy. Informed consent was obtained according to each institution's guidelines.

Based on phase I study data,[10] asaley was given at a dose of 800 mg/m²/day orally in four divided doses for a 4-day course at monthly intervals. The patient's status during therapy was evaluated by physical examination, peripheral blood counts, and bone marrow examinations. An adequate trial was defined as one course of therapy with a 4-week survival in patients with progressive disease and two courses of therapy in patients with improved or nonprogressive disease.

RESULTS

Thirty-five patients were eligible for study. Twenty-nine were fully evaluable for both objective response and toxicity. The median age of these patients at diagnosis was 57 months (range, 13–164 months). The median time from diagnosis to treatment with asaley was 26 months.

Five patients had an inadequate trial because of early death and an assessment of objective response could not be made. One was not evaluable because a low dose of medication was given.

RESPONSE

Twenty-five patients received one course of therapy and all demonstrated progressive disease by either a rise in the number of blast cells in the bone marrow, a rise in the peripheral blood counts, or increasing hepatosplenomegaly.

Four patients received two courses of chemotherapy. One of the four had a complete response with a normal physical examination, peripheral blood counts, and bone marrow examination 30 days after the second course of asaley was given. The remaining three patients demonstrated progressive disease.

TOXICITY

Moderate nausea and vomiting controllable with antiemetics occurred in 14 of 29 patients (48%).

[1]Received Dec 27, 1976; revised Apr 1, 1977; accepted Apr 12, 1977.

[2]Supported by Public Health Service grants CA-11233, CA-16388, CA-03713, CA-08223, CA-16943, and CA-12014 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3]This study was conducted by the Southwest Oncology Group.

[4]Asaley: NSC-167780; CAS reg. No. 3577-89-7; L-leucine, *N*-[*N*-acetyl-4-[bis(2-chloroethyl)amino]-DL-phenylalanyl]-, ethyl ester.

[5]Division of Pediatric Hematology-Oncology, University of Oklahoma Health Sciences Center, Oklahoma City.

[6]Medical University of South Carolina, Charleston.

[7]University of Texas System Cancer Center, Houston.

[8]University of Virginia School of Medicine, Charlottesville.

[9]Regulations for the Production of Asaley. An NIH library translation of the product information brochure from the Soviet Union (NIH 73-132-C).

*Reprint requests to: C. L. Sexauer, MD, Division of Pediatric Hematology-Oncology, The University of Oklahoma Health Sciences Center, PO Box 26901, Oklahoma City, Okla 73190.

[10]Gottlieb JA. Data presented at the Southwest Oncology Group meeting. Oklahoma City, Okla, Oct 8–11, 1974.

Severe granulocytopenia (< 500 cells/mm^3) occurred in nine of 29 patients (31%) and severe thrombocytopenia (< 50,000 cells/mm^3), clearly not attributable to increasing disease, occurred in five of 29 patients (17%). The mean time to the nadir of granulocytopenia was 11 days and to the nadir of thrombocytopenia was 13 days.

CONCLUSIONS

Under the conditions of this study, asaley does not appear to be significantly active as an induction agent in the treatment of childhood ALL.

REFERENCES

1. LARIONOV LF, BUKHAROVA IK, and SHANAYEVA YM. Experimental studies of the toxicity and anti-neoplastic activity of the ethyl ester of acetyl-sarcolysin-L-leucine (asaley). Vopr Onkol 11:78–80, 1965.
2. ZAYTSEVA LA, BOVODKINA RP, LOPATIN PV, ET AL. The antineoplastic effect of asalin and asaley with course wise and single enteral, intramuscular and rectal administration. Pharmakol Toksikol 4:460–463, 1971.

Chemotherapy of Metastatic Gastrointestinal Neoplasms With 5-Fluorouracil and Streptozotocin[1,2]

Brief Reports and Preliminary Communications

Mark Seligman, Ronald M. Bukowski,* Carl W. Groppe, Jr, James K. Weick, James S. Hewlett, and Richard L. Greenstreet[3]

Following the introduction of 5-fluorouracil (5-FU) in 1957, its use in advanced gastrointestinal (GI) carcinomas was extensively investigated. As a single agent it proved to have only limited usefulness (1). Streptozotocin is an antibiotic of unique chemical structure derived from the *Streptomyces achromagenes*, and possesses a nitrosourea moiety. It has attracted special interest because of the minimal myelotoxicity associated with its use. Its predominant side effects are reversible renal, hepatic, and pancreatic β cell toxicity (2). The present study was designed to determine the response rates of various GI neoplasms to the combination of streptozotocin and 5-FU.

MATERIALS AND METHODS

Fifty-one patients with biopsy-proven carcinoma of the GI tract were studied. Patient characteristics are listed in table 1. All patients were ambulatory, had an estimated life expectancy of > 6 weeks, and had measurable disease parameters. None of the patients had prior chemotherapy or had received radiation therapy for ≥ 1 month prior to the start of treatment. Informed consent was obtained from all patients.

Chemotherapy consisted of a 5-day intensive course of 5-FU and streptozotocin, both given at a dose of 500 mg/m²/day iv by rapid push every 28 days. Patients with a history of extensive radiation, bone marrow involvement by tumor, or serum bilirubin > 3.0 mg/100 ml, or those > 65 years of age

[1]Received Jan 3, 1977; revised May 23, 1977; accepted May 25, 1977.

[2]5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8.
Streptozotocin: NSC-85998.

[3]Department of Hematology and Medical Oncology, The Cleveland Clinic Foundation, Ohio.

*Reprint requests to: Ronald M. Bukowski, MD, The Cleveland Clinic Foundation, 9500 Euclid Ave, Cleveland, Ohio 44106.

TABLE 1.—Patient characteristics and response data

	Tumor type			
	Pancreas	Gastric	Colorectal	Miscellaneous*
No. of patients	19	12	14	6
Age (mean)	59	56	61	53
Performance status (Karnofsky)				
9–10	2	4	4	1
7–8	15	5	9	4
5–6	2	3	1	1
Site of measurable lesion				
Liver	6	4	9	1
Pulmonary	1	1	4	0
Abdominal	12	7	1	5
Responses (complete and partial)	4	4	2	2
MST† (days)				
Responders	334‡	384‡	353	—
Nonresponders	76	177	118	—

*Carcinoid, islet cell, biliary carcinoma.
†Median survival time.
‡Significant difference for responders vs nonresponders, $P < 0.05$.

received 300 mg/m²/day of 5-FU and streptozotocin. Cbc and platelet counts were obtained every 14 days, and liver and kidney functions were determined every 28 days.

A complete response was defined as the absence of clinically detectable tumor. A partial response was defined as a decrease of 50% in the product of the two diameters of any measurable lesion, a 30% reduction of the sum of liver measurements below the right and left costal margins and xiphoid process, no increase in the size of other lesions, and no decrease in weight or performance status (Karnofsky scale). Stable disease was defined as insufficient regression to be a partial response with no new lesions. Progressive disease was defined as > 25% increase in the product of the diameters of any measurable lesion, or the appearance of new lesions. Fifty-seven patients were initially entered in the study; four were considered unevaluable because of failure to complete one course of treatment and two were unevaluable because of lack of measurable disease. Survival data were analyzed using the Kaplan-Meier (3) survival curves. Statistical analyses of differences in survival were performed by the method of Thomas et al (4) using a generalized K/W analysis (Gehan-Breslow) and a National Cancer Institute computer program (Trend and Homogeneity Analyses of Proportions and Life Table Data, version 8/10/76).

RESULTS

Objective responses were noted in 12 of 51 patients (23.5%). The response data and survival for all evaluable cases are outlined in table 1. Four of 19 patients with adenocarcinoma of the exocrine pancreas demonstrated objective responses. The sites of disease in these four patients were abdominal masses in three and liver involvement in one. The four responding patients did demonstrate a significant increase in survival when compared to the nonresponders ($P < 0.03$). Four of 12 patients with gastric adenocarcinoma demonstrated a response. The sites of disease in the four responders were two abdominal, one liver, and one pulmonary.

As in the group of patients with pancreatic adenocarcinoma, the responders had a significant increase in survival when compared to the nonresponders ($P < 0.05$). Only two of 14 patients with metastatic colorectal carcinoma demonstrated a response. Finally, an objective response was noted in a patient with metastatic carcinoid and another in a patient with a nonfunctioning islet cell tumor.

The toxicity encountered in this study was moderate. Moderately severe nausea and vomiting occurred in 86% of the patients. Thirty-five percent of the patients demonstrated a wbc count < 3000/mm³ or a platelet count < 100,000/mm³, necessitating a dose reduction in the subsequent courses of treatment, and in nine instances hematologic toxicity was severe with the wbc count dropping to < 1500/mm³ and the platelet count to < 50,000/mm³. The blood counts were obtained only every 14 days, and, therefore, the myelotoxicity in this study may be underestimated. Other side effects included hyperglycemia (24%), mucositis (22%), proteinuria (8%), aminoaciduria (2%), and elevations of BUN (2%). Generalized increased pigmentation developed in two patients who had been receiving chemotherapy for > 6 months. One death directly attributable to the drug combination occurred and was secondary to the inadvertent administration of 8 g of streptozotocin with resulting anuria and severe hepatotoxicity.

DISCUSSION

The response rate in pancreatic cancer to 5-FU and streptozotocin was 21%. The overall response rates to 5-FU and streptozotocin as single agents against pancreatic cancer are reported to be 20% (5) and 33% (6) respectively, and appear similar to the combination of these two agents in this study. In gastric adenocarcinoma the response rate was 33%. The overall objective response rate to 5-FU as a single agent is 27% (7), and the rate of the present drug combination appears similar to this. The exact response rate to the combination of 5-FU and streptozotocin in gastric carcinoma remains to be determined; however, the combination of 5-FU with a minimally myelosuppressive nitrosourea is advantageous in that other drugs such as mitomycin C or adriamycin can perhaps be added to this combination without additive toxicity. The response rate in colon cancer was unremarkable, and effectiveness in metastatic carcinoid and islet cell neoplasms remains to be determined. The combination of 5-FU and streptozotocin is a well-tolerated regimen with only moderate toxicity. If response rates found in patients with adenocarcinoma of the stomach can be confirmed, then the study of additional combinations including mitomycin C and/or adriamycin in this neoplasm may be justified.

REFERENCES

1. MOERTEL CG, and REITEMEIER RJ. Chemotherapy of gastrointestinal cancer. Surg Clin North Am 47:929-953, 1967.
2. MOERTEL CG, REITEMEIER RJ, SCHUTT AJ, ET AL. Phase II study of streptozotocin (NSC-85998) in the treatment of advanced gastrointestinal cancer. Cancer Chemother Rep 55:303-307, 1971.

3. KAPLAN EL, and MEIER P. Non-parametric estimation from incomplete observations. J Am Stat Assoc 53:457–481, 1958.
4. THOMAS DG, BRESLOW N, and GART JJ. Trend and homogeneity analyses of proportions. In press.
5. MACDONALD JS, WIDERLITE L, and SCHEIN PS. Current diagnosis and management of pancreatic carcinoma. J Natl Cancer Inst 56:1093–1099, 1976.
6. CARTER SK, and COMIS R. Adenocarcinoma of the pancreas: current therapeutic approaches, prognostic variables, and criteria of response. *In* Cancer Therapy: Prognostic Factors and Criteria of Response (Staquet MJ, ed), New York, Raven Press, 1975, pp 237–253.
7. MOERTEL CG. Carcinoma of the stomach: prognostic factors and criteria of response to therapy. *In* Cancer Therapy: Prognostic Factors and Criteria of Response (Staquet MJ, ed). New York, Raven Press, 1975, pp 229–236.

Phase II Study of β-2′-Deoxythioguanosine in Adult Acute Leukemia[1,2,3]

G. A. Omura,* W. R. Vogler, R. V. Smalley, N. Maldonado, G. O. Broun, W. H. Knospe, Y. S. Ahn, and G. B. Faguet[4]

Writing Committee for the Southeastern Cancer Study Group

β-2′-Deoxythioguanosine (β-TGdR) is a purine analog which has been highly active in some animal tumor systems resistant to 6-thioguanine[5] (6TG) (1) but not in others (2). The greatest % increase in lifespan in animal systems resulted from a daily × 5 schedule.[6] A 5-day course repeated q2wks has been evolved for clinical trials (3,4). The Southeastern Cancer Study Group (SEG) has tested β-TGdR to determine its antileukemic activity.

MATERIALS AND METHODS

β-TGdR was supplied as a lyophilized powder in 50- and 200-mg vials. It was reconstituted with sterile water or saline. Patients who were ≥ 15 years old with acute leukemia were eligible after written consent. Except as noted, all patients were to be in relapse but were to have recovered from the toxicity of prior treatment. Patients with a blastic transformation of chronic myelogenous leukemia (BTL) were eligible without prior acute leukemia therapy. The initial starting dose was 300 mg/m²/day × 5 by rapid iv injection. On Day 15, the dose was escalated to 400 mg/m²/day × 5 and subsequent courses q2wks were increased to 500 mg/m²/dose, if marrow hypoplasia or remission was not observed at lower doses. After the initial group at the 300-mg/m² starting dose had been evaluated with no significant toxicity (marrow aplasia) from the first course, the starting dose was raised to 400 mg/m². Remissions were to be maintained on 3–5-day courses of the drug q3wks. SEG criteria (5) for response and for toxicity (6) were used. The grade of hematologic toxicity (1+ or 2+) is related to the magnitude of the fall rather than any absolute level. Grade 3 toxicity is a life-threatening complication associated with blood count depression. An evaluable patient is one who is eligible for study and treated according to protocol. An adequate trial was defined as three courses (starting at the revised dose level of 400 mg/m²) given on schedule unless marrow hypoplasia required a delay.

RESULTS

Response

The data are presented in table 1. Forty-six patients (26 with acute myelocytic leukemia [AML], 16 with BTL, and four with acute lymphocytic leukemia [ALL]) were initially treated at the start-

[1]Received Jan 11, 1977; revised May 16, 1977; accepted May 25, 1977.

[2]β-2′-Deoxythioguanosine: NSC-71261; 9*H*-purine-6-thiol, 2-amino-9-(2-deoxy-β-D-erythro-pentofuranosyl)-, monohydrate. Supplied by the Division of Cancer Treatment, National Cancer Institute (NCI), Bethesda, Md.

[3]This study was performed by the Southeastern Cancer Study Group (SEG) and was supported by Public Health Service grants (CA) from the NCI, National Institutes of Health, Department of Health, Education, and Welfare. The following members of the SEG participated in the study: *J. Carpenter, M. Conrad, J. Durant, R. Gams, D. Lineberry,* and *G. Omura* (University of Alabama, Birmingham, CA-03013); *H. Silberman* and *D. S. Miller* (Duke University, Durham, NC, CA-03177); *W. B. Kremer* (Durham Veterans' Administration [VA] Hospital, NC, CA-05634); *E. Bruckner, L. Cooper, C. Corley, J. Hardison, C. Huguley, Jr, J. Keller, J. Schmale, C. Vogel, W. R. Vogler,* and *W. Whaley* (Emory University, Atlanta, Ga, CA-03227 and CA-11263); *G. Faguet* (Medical College of Georgia, Augusta, CA-06807); *Y. Ahn* and *H. Lessner* (University of Miami, Fla, CA-05641); *D. Gorshein* and *S. Murphy* (University of Pennsylvania, CA-12639); *W. Barry, R. Joseph,* and *R. Smalley* (Temple University, Philadelphia, Pa, CA-07961); *V. Loeb, Jr, C. Presant, E. Reinhard,* and *S. Safdar* (Washington University, St. Louis, Mo, CA-03376); *E. Velez-Garcia* and *N. Maldonado* (University of Puerto Rico, San Juan, CA-12223); *S. A. Gregory* and *W. Knospe* (Rush-Presbyterian St. Luke's Medical Center, Chicago, Ill. CA-12640); *S. Krauss* (University of Tennessee, Knoxville, CA-13237); *K. Tornyos,* New Orleans VA Hospital, La, CA-13249); *R. W. Kellermeyer* and *A. Rassiga* (Case Western Reserve University, Cleveland, Ohio, CA-15584); *G. Porter* and *D. Samples* (Ochsner Clinic, New Orleans, La, CA-15578); *C. Neely* (University of Tennessee, Memphis, CA-17027); *G. O. Broun, Jr* (St. Louis University, Mo, CA-17214); *P. Lavik* and *K. Rehman* (Brooklyn VA Hospital, NY).

[4]We gratefully acknowledge the assistance of Dr. A. Bartolucci and the staff of the Statistical Office of the SEG, and the secretarial assistance of C. D. Marks.

[5]6-Thioguanine: NSC-752; CAS reg. No. 1832-72-0; purine-6-thiol, 2-amino-.

[6]Rosenbaum C, and Carter SK. Clinical brochure: β-TGdR. Prepared by the NCI, Bethesda, Md, Oct 1969.

**Reprint requests to*: George A. Omura, MD, University of Alabama Medical Center, University Station, Birmingham, Ala 35294.

TABLE 1.—Response to β-TGdR

Disease	Dose* (mg/m²)	No. of patients: Entered	Not evaluable†	Evaluable	No. of patients receiving— < 3 courses: Died	Worse	NC‡	< PR	PR	≥ 3 courses: Died	Worse	NC‡	< PR	PR	CR
AML	300	26	7 PV, 2 other	17	7	1	0	0	0	1	2	2	3	0	1
	400	66	12 PV, 5 other	49	12	2	1	1	2	6	7	11	1	0	6
BTL	300	16	5 PV	11	6	0	0	1	0	0	0	2	2	0	0
	400	41	10 PV, 5 other	26	7	1	1	0	0	3	2	6	3	0	3
ALL	300	4	0	4	2	0	0	0	0	0	0	0	1	1	0
	400	12	2 PV	10	3	1	0	0	0	2	2	0	1	0	1

*300 = starting dose level.
†PV = protocol violation; other = ineligible (wrong diagnosis), intercurrent disease, or refused therapy.
‡ NC = no change.

TABLE 2.—Hematologic toxicity in patients evaluable for toxicity

Dose (mg/m²)	Granulocytopenia: Grade 1+	2+	3+	Thrombocytopenia: Grade 1+	2+	3+
300 (44 patients)	5(11%)	15(34%)	7(16%)	8(18%)	6(14%)	2(5%)
400 (111 patients)	13(12%)	36(32%)	11(10%)	14(13%)	29(26%)	3(3%)

ing dose of 300 mg/m². In this group, there was only one complete response (CR) (duration, 49 days) in a patient with AML with major prior treatment, including 6TG. Of the ALL patients, one achieved a partial remission (PR) for 6 months (all parameters returned to normal except splenomegaly which persisted); he had received prior treatment with 6TG.

Sixty of 66 AML patients started at a dose of 400 mg/m² had had prior therapy. Only two previously treated patients had not received 6-mercaptopurine or 6TG. Six patients achieved CR and two achieved PR. Three patients who achieved CR (remission duration, 3, 4, and 5 months) had had extensive prior treatment with 6TG. Of the six previously untreated patients, three achieved CR (remission duration 5, 6, and 7 months). All of the responding patients developed a hypocellular marrow before achieving CR and required at least three induction courses (median, three courses).

Three BTL patients achieved CR (duration, 30, 41, and 51 days). Thirty-nine had had busulfan during the chronic phase and 12 had had prior purine analog therapy.

One ALL patient who had not had purine analogs before transiently qualified as a complete responder and one patient had a response that was less than a PR.

Toxicity

Table 2 summarizes the hematologic toxicity. The major effect was on granulocytes. There was one drug death which occurred at the 300-mg/m² dose level. At the 400-mg/m² dose level, skin toxicity was manifested in 10% of the cases (11 of 111 cases) as a maculopalpular erythematous rash which, in some cases, seemed to be exacerbated by sunlight and was transient. Alopecia occurred in 16% of the cases (18 of 111) but was reversible, stomatitis was described in 8% (nine of 111), and nausea and vomiting were minor side effects in 15% (17 of 111) and were major in 4% (four of 111). Abnormalities of liver function tests were seen in 6% of the patients, but there were alternative explanations in those patients.

DISCUSSION

Bodey et al (5) reported 19 adequate trials of β-TGdR in which there were two CRs and four PRs in acute leukemia using slightly lower drug doses.

The present trial confirms that the drug is well tolerated, that its major toxicity is hematologic (especially on granulocytes), and that remissions can be achieved in acute leukemia. With the excep-

tion of the six AML patients who had had no prior therapy (three of whom achieved CR), all other patients either had been extensively pretreated or had a form of leukemia (BTL) which is generally considered to be quite refractory to standard therapy. β-TGdR appears to be an active antileukemic agent and warrants further study.

REFERENCES

1. LEPAGE GA, JUNGA IG, and BOWMAN B. Biochemical and carcinostatic effects of 2′-deoxythioguanosine. Cancer Res 24:835–840, 1964.
2. NELSON JA, KUHNS JN, and CARPENTER JW. Lack of activity of beta-2′-deoxythioguanosine against two tumors resistant to 6-thioguanine. Cancer Res 35:1372–1374, 1975.
3. OMURA GA. Phase II trial of beta-deoxythioguanosine (β-TGdR, NSC 71261) in refractory adult acute leukemia. Proc Am Assoc Cancer Res and ASCO 16:140, 1975.
4. BODEY GP, MCCREDIE KB, WHITECAR JP, ET AL. Clinical studies of beta-thioguanine deoxyriboside alone and in combination with arabinosyl cytosine. Med Pediatr Oncol 2:199–205, 1976.
5. VOGLER WR, HUGULEY CM, and RUNDLES RW. Comparison of methotrexate with 6-mercaptopurine-prednisone in treatment of acute leukemia in adults. Cancer 20:1221–1226, 1967.
6. KREMER W, VOGLER WR, and CHAN YK. An attempt at synchronization of marrow cells in acute leukemia—relationship to therapeutic response. Cancer 37:390–403, 1976.

Adriamycin Versus Adriamycin and Bleomycin in Advanced Epidermoid Carcinoma of the Cervix [1,2,3]

Brief Reports and Preliminary Communications

Bernard R. Greenberg,[4,*] Carl G. Kardinal,[5] Thomas F. Pajak,[6,7] and Joseph R. Bateman [8,9]

The treatment of recurrent or metastatic epidermoid carcinoma of the cervix remains a difficult therapeutic problem. A beneficial influence of single agents on survival has not been adequately demonstrated. Recent studies, although preliminary, reveal an improved response frequency with drug combinations such as methotrexate and bleomycin (1,2); vincristine, bleomycin, and mitomycin C (3); and adriamycin and bleomycin (4). The combination of adriamycin and bleomycin is attractive because of its relative ease of administration and potential lack of additive toxicity. This randomized study was undertaken to compare this combination to adriamycin alone.

MATERIALS AND METHODS

Patients with recurrent or metastatic epidermoid carcinoma of the cervix were entered in the study if they had measurable disease parameters by physical examination, roentgenographic studies, or radioisotope scans. Pretreatment observations also included a complete history and physical examination with baseline laboratory studies. Patients were seen weekly with a followup physical examination and repeated laboratory studies as necessary for response and toxicity monitoring.

Patients were stratified prior to entry in the study according to two variables believed to be of major influence on either response or toxicity. These stratifying variables were the extent of disease (only pelvic disease versus distant metastases) and prior anticancer therapy (none versus any). Patients were randomized within each stratum by the Statistical Office of the Western Cancer Study Group to regimen A (adriamycin alone) or to regimen B (adriamycin and bleomycin). Twenty patients (regimen A, nine patients and regimen B, 11 patients) were entered and their pretreatment characteristics are summarized in table 1.

All patients with prior pelvic radiation therapy received escalating adriamycin doses in order to prevent prohibitive hematologic toxicity. The first course was 30 mg/m^2 iv, the second course was 45 mg/m^2, and the third and further courses were 60 mg/m^2 if no significant hematologic toxicity developed. The adriamycin courses were administered

TABLE 1.—Summary of pretreatment characteristics and responses

	Regimen A: adriamycin alone	Regimen B: adriamycin and bleomycin
No. of patients entered	9	11
Prior chemotherapy	1	2
Prior radiotherapy	9	11
Pelvic recurrences only	0	5
Pelvic recurrences with distant metastases	7	6
Distant metastases only	2	0
No. of partial responses in metastatic disease	5	0
No. of partial responses in pelvic disease	0	0

[1]Received Jan 21, 1977; revised May 23, 1977; accepted May 25, 1977.

[2]Supported in part by Public Health Service grants CA-14450, CA-06800, CA-14472, and CA-05186 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3]Adriamycin: NSC-123127; CAS reg. No. 23214-92-8.

Bleomycin: NSC-125066; CAS reg. No. 11056-06-7; [2,4'-bithiazole]-4-carboxylic acid, 2'-(2-aminoethyl)-, monohydrate.

[4]Department of Internal Medicine, Section of Hematology and Oncology, University of California School of Medicine, Davis.

[5]Department of Internal Medicine, Ellis Fischel State Cancer Hospital, Columbia, Mo.

[6]Department of Medicine, University of Southern California, Los Angeles, and Biostatistician, Western Cancer Study Group.

[7]*Present address*: Cancer and Leukemia Group B, Scarsdale, NY.

[8]Department of Medicine, Division of Medical Oncology, University of Southern California, Los Angeles.

[9]This study was done by the Western Cancer Study Group. The following institutions participated in the study: University of California, Davis (*J. P. Lewis*, CA-14450); Ellis Fischel Cancer Hospital, Columbia, Mo (*C. Kardinal*, CA-06800); John Wesley County Hospital, Los Angeles, Calif (*J. Bateman*, CA-05186); University of California, Irvine (*S. Armentrout*, CA-14472); US Air Force Hospital, Travis Air Force Base, Calif (*R. Reynolds*, CA-05186); Letterman General Hospital, San Francisco, Calif (*M. Corder*, CA-05186).

[*]*Reprint requests to*: Bernard R. Greenberg, MD, Department of Internal Medicine, Section of Hematology and Oncology, 4301 X St, Sacramento, Calif 95817.

every 3 weeks if not prevented by residual toxicity. Patients in regimen B additionally received iv bleomycin once a week at a dose of 10 units/m^2.

RESULTS

All patients were considered to be evaluable for response, but only 19 were considered for toxicity evaluation. Five partial responses (50% regression) in metastatic sites were observed in the nine patients entered in regimen A (table 1). Two other patients had stabilization of their metastatic disease for 6 weeks and 5 months. None of the 11 patients in the combination regimen showed any responses in metastatic sites except one patient with disease stabilization. No responses in previously irradiated pelvic lesions were seen with either regimen. None of the three patients with prior chemotherapy responded. There was a statistically significant higher response frequency associated with adriamycin alone ($P = 0.026$), but there was no difference in survival between the two regimens (4.0 versus 4.3 months) or between patients who showed response or disease stabilization and nonresponding patients.

TOXICITY

Fourteen patients eventually received the full dose of adriamycin. Five patients remained at the initial starting dose of 30 mg/m^2 (regimen A, two patients and regimen B, three patients) and one received ≤ 45 mg/m^2 (regimen B). The commonly observed toxicities were nausea and vomiting and alopecia. Two patients developed probable bleomycin pulmonary toxicity as judged by both physical examination and chest roentgenograms after 34 and 150 units. There were no instances of severe thrombocytopenia and in only one patient was a moderate fall in the platelet count observed (60,000 cells/mm^3). In the majority of the patients there was either none or only mild neutropenia. The nadir granulocyte count was $< 1000/mm^3$ in only two patients (788/mm^3 and 437/mm^3). There were no instances of life-threatening infections or bleeding other than those attributable to local tumor growth. No adriamycin cardiotoxicity was observed.

DISCUSSION

Recent interest has been shown in the role of combination chemotherapy in advanced cervical carcinoma. The combination of adriamycin and bleomycin was found to be effective by Monfardini et al (4), and superior to the combination of cyclophosphamide and vincristine. This is in contrast to the study of Barlow et al (5), in which this combination was found to be inferior to adriamycin alone in epidermoid carcinoma of the cervix. These conflicting results prompted this present randomized study. The five partial responses were all observed in patients who received adriamycin alone (table 1) and were in nonirradiated metastatic lesions: pulmonary (three patients), liver (one), para-aortic node (one), and abdominal mass (one). In fact, it was common to see regression of distant metastases with no effect on pelvic lesions. These responses were observed early but unfortunately were of short duration. No significant effect on overall survival was observed between the two regimens or between the responders and the nonresponders.

The results of this study support the previous observations of Barlow et al (5). The ineffectiveness of this combination cannot be attributed to a reduced dose of adriamycin and may represent an inhibitory effect of bleomycin on adriamycin's activity in this particular malignancy. The dose of bleomycin used in this study is considered low, however, and possibly higher doses administered intermittently or by continuous infusion might add to the response frequency observed with adriamycin alone. Though pelvic recurrences in previously irradiated areas were not affected by adriamycin, a satisfactory response frequency (56%) was observed in metastatic lesions. Adriamycin may thus prove effective in eliminating microscopic, clinically inapparent disease when administered after the initial surgical procedure or primary course of radiation therapy in patients at high risk for recurrence. Further studies to evaluate its role as adjuvant therapy in patients with stage IIB, IIIA, and IIIB disease appear indicated.

REFERENCES

1. CONROY JF, LEWIS GC, BRADY LW, ET AL. Low dose bleomycin and methotrexate in cervical cancer. Cancer 37:660–664, 1976.
2. PREL FJ, SLAYTON RE, PERLIA CP, ET AL. Combination chemotherapy with bleomycin and methotrexate in recurrent and disseminated cervical carcinoma: a preliminary study. Gynecol Oncol 1:184–190, 1975.
3. BAKER LH, OPIPARI MI, and IZBICKI RM. Phase II study of mitomycin-C, vincristine, and bleomycin in advanced squamous cell carcinoma of the uterine cervix. Cancer 38:2222–2224, 1976.
4. MONFARDINI S, DE PALO GM, BAJETTA E, ET AL. Adriamycin (ADM) plus bleomycin (BLM) versus CTX plus VCR in advanced carcinoma of the cervix. Proc Am Assoc Cancer Res and ASCO 15:361, 1974.
5. BARLOW JJ, PIVER MS, CHUANG JT, ET AL. Adriamycin and bleomycin alone and in combination in gynecologic cancers. Cancer 32:735–743, 1973.

Adriamycin Therapy in American Patients With Hepatocellular Carcinoma[1,2]

Brief Reports and Preliminary Communications

Daniel C. Ihde,* Robert C. Kane, Martin H. Cohen, K. Robert McIntire, and John D. Minna[3,4]

Hepatocellular carcinoma is rarely diagnosed at a time when surgical resection is possible (1,2). A median survival from diagnosis of 2–4 months is reported in the US (3) and Africa (2). Radiotherapy is not an effective treatment modality (2); hence, most patients are candidates for chemotherapy at the time of diagnosis. Some responsiveness of this tumor to both iv (4) and intra-arterial (3) agents has been reported. However, in South African patients, prospective randomized comparisons of numerous drugs to placebo have demonstrated improved survival only with intra-arterial methotrexate (2).

Therefore, the initial findings from Uganda of objective responses in 11 of 14 hepatoma patients to the anthracycline antibiotic adriamycin (ADM) (5) were exciting. Prompted by these results, we initiated a trial of ADM therapy for American hepatoma patients.

PATIENTS AND METHODS

Thirteen male patients with a median age of 58 years (range, 18–69 years) were treated. All had a histologically confirmed diagnosis of hepatocellular carcinoma, measurable or evaluable tumor masses, and a peripheral wbc count of $> 4000/\mu l$ and platelet count of $> 100{,}000/\mu l$ at the start of therapy. Other pretreatment characteristics are shown in table 1. Among the four patients with good performance status, none had jaundice and only one was known to have cirrhosis. Before and during the therapy of nine patients, serum was obtained for determinations of alpha-fetoprotein (AFP) by radioimmunoassay (6) a median of seven times.

TABLE 1.—Characteristics of 13 adriamycin-treated patients

Characteristic	No. of patients
Jaundice (bilirubin > 2.0 mg/dl)	6
Extrahepatic metastases	4
Performance status	
Fully ambulatory	4
Partially bedridden	9
Cirrhosis	9/10*
Chronic alcoholism	7
Ascites	3
Weight loss > 10% (> 20%)	10(2)
Tumor differentiation	
Well and moderately well	10
Poor and anaplastic	3
Prior therapy	
Hepatic lobectomy	1
Chemotherapy	1
Elevated AFP	7†
Hepatitis B antigen detected	3/12*
Tumor in bone marrow biopsy	1/9*

*No. positive/No. evaluable.

†6 of 10 by radioimmunoassay (ref 6) and 1 of 3 by qualitative commercial test.

ADM was given iv in a dose of 60 or 75 mg/m² to patients with a serum bilirubin of < 3.0 mg/dl. The latter dose was used in 15% of the courses. With a higher serum bilirubin, the dose was reduced to 45 mg/m². The drug was administered every 3 weeks until tumor progression or death occurred or until a total dose of > 500 mg/m² had been reached. Three patients had treatment discontinued at that point, and were observed without further therapy until relapse. Peripheral blood cell counts were determined one or more times during the 3 weeks after a dose of ADM. Therapy was delayed at 3 weeks if recovery of blood cell counts had not occurred.

A partial response (PR) was defined as a 50% decrease in the area of the most clearly measurable mass lesion or a 30% decrease in the sum of liver measurements below each costal margin and the xiphoid (either was required to persist for at least 1 month in the absence of progressive tumor else-

[1]Received Jan 21; 1977; revised Mar 31, 1977; accepted Apr 20, 1977.

[2]Adriamycin: NSC-123127; CAS reg. No. 23214-92-8.

[3]National Cancer Institute (NCI)-Veterans' Administration (VA) Medical Oncology Branch, VA Hospital, Washington, DC, and the Division of Cancer Biology and Diagnosis, NCI, Bethesda, Md.

[4]We wish to thank Dr. Hyman J. Zimmerman, Medical Service, VA Hospital, Washington, DC, for advice during the design of the study and for reviewing the manuscript.

*Reprint requests to: D. C. Ihde, MD, NCI-VA Medical Oncology Branch, VA Hospital, 50 Irving St, NW, Washington, DC 20422.

where). Major hematologic toxicity was defined as a wbc count $< 2500/\mu l$ and/or a platelet count $< 50,000/\mu l$. Survival was calculated from the start of therapy.

RESULTS

Response and survival.—Only two of the 13 patients (15%) treated with ADM had a PR; these responses lasted 5+ and 8 months. Both responding patients received > 500 mg/m² of ADM, and the third patient given a full course of treatment had stable disease while receiving therapy and has now survived 21+ months without evidence of disease progression. In three patients, minor antitumor effects (either a transient PR or improvement in liver function tests and performance status) were noted but these did not appear to affect survival. Median survival of all patients was 3 months, with a range of 1–21+ months.

Of all pretreatment characteristics in these patients, performance status was the best predictor of both survival and the possibility of achieving a PR (*see* table 2). The only responses occurred in the good performance status patients, in whom the median survival will exceed 12 months; no PRs were noted in the poor performance status patients, whose median survival was 2 months.

TABLE 2.—Relationship of performance status to response and survival

Performance status	No. of patients with PR/total No. treated	Median survival (mos)/range
Fully ambulatory	2/4	12+/3–21+
Partially bedridden	0/9	2/1–4

Toxicity.—Major hematologic toxic effects were observed in only four patients, with suppression of wbc counts in all four patients and of platelets in one patient. There was one drug-related death from leukopenia and sepsis. Of 36 doses of ADM (60 or 75 mg/m²) given to patients with serum bilirubin < 3.0 mg/dl, major leukocyte toxicity (as defined above) was seen after only two doses and comparable platelet toxicity after none. One patient developed controllable congestive heart failure 1 month after completing a full course of ADM. Emesis, stomatitis, alopecia, and hyperpigmentation of nails were also seen.

Serial AFP determinations.—Initial AFP values were normal (< 40 ng/ml) in three patients and elevated (range, 64–1,275,000 ng/ml) in six patients. Both patients with a PR had normal initial AFP levels which remained normal throughout their periods of observation despite eventual tumor regrowth in one case. Of the six patients with elevated AFP values before treatment, two showed evidence of minor antitumor effects (transient tumor shrinkage and improved performance status) after the first dose of ADM. In one of these patients, there was a simultaneous drop in serum AFP to one third of the pretreatment level. All of the six patients with initially elevated AFP died with rising AFP values, often accompanied by objective tumor growth.

DISCUSSION

The 15% response rate to ADM seen in our patients with hepatocellular carcinoma does not equal that reported by Olweny et al (5), but is not greatly dissimilar from the experience of two other groups studying larger numbers of American and African hepatoma patients (7,8). In our study, hematologic toxicity was mild to moderate, but it is doubtful that our lower response rate is due to failure to always administer the dose of 75 mg/m² used in Olweny's (5) patients since Vogel et al (8) obtained only 4 PRs among 16 patients given the larger dose of ADM.

The discrepancy is also unlikely to result from a greater responsiveness of African hepatoma patients to ADM. Results of chemotherapy in African patients were comparable or less successful than those noted in American patients in the studies of Vogel et al (8) and Falkson et al (7). It could conceivably be related to varying definitions of partial responsiveness. Olweny's group (5) did not specify a minimum duration for their PRs, and both we and Vogel et al (8) have more than rarely observed transient diminutions in tumor size in patients who were not considered to have achieved a PR.

Though based on a small number of observations, our finding that the patient's performance status is a good predictor of both response to chemotherapy and survival in this disease is consistent with other recent studies. In the ADM trial of Vogel et al (8), patients without jaundice and with good performance status had a higher response rate than those not in this "good risk" group. Improved survival was seen in patients without jaundice or cirrhosis and with ambulatory performance status in the Eastern Cooperative Oncology Group study of Falkson et al.[5] In our patients, of course, these factors were often associated.

[5]Falkson G. Chemotherapy of primary liver carcinoma. Presented at the 67th Annual Meeting of the American Association for Cancer Research, Toronto, Canada, May 1976.

In this study, serum AFP paralleled the course of advancing disease in most instances, as previously demonstrated in African patients (6). Elevated AFP levels will diminish with response to chemotherapy (5), as shown transiently in one of our patients.

Despite the modest response rate reported here for patients given ADM, it is encouraging that this drug has demonstrated activity against hepatocellular carcinoma in four trials. The combination of ADM with other drugs or therapeutic modalities would seem worthy of investigation in this prognostically bleak disease.

REFERENCES

1. CADY B, and OBERFIELD RA. Arterial infusion chemotherapy of hepatoma. Surg Gynecol Obstet 138:381–384, 1974.
2. FALKSON G. Therapeutic approaches to hepatoma. Cancer Treat Rev 2:73–76, 1975.
3. AL-SARRAF M, GO TS, KITHIER K, ET AL. Primary liver cancer: a review of the clinical features, blood groups, serum enzymes, therapy, and survival of 65 cases. Cancer 33:574–582, 1974.
4. MOERTEL CG. Clinical management of advanced gastrointestinal cancer. Cancer 36:675–682, 1975.
5. OLWENY CLM, TOYA T, KATONGOLE-MBIDDE E, ET AL. Preliminary communication: Treatment of hepatocellular carcinoma with adriamycin. Cancer 36:1250–1257, 1975.
6. MCINTIRE KR, VOGEL CL, PRIMACK A, ET AL. Effect of surgical and chemotherapeutic treatment on alpha-fetoprotein levels in patients with hepatocellular carcinoma. Cancer 37:677–683, 1976.
7. FALKSON G, MOERTEL CG, and LAVIN PT. Chemotherapy of primary liver carcinoma: a parallel study in American and African Bantu patients. Proc Am Assoc Cancer Res and ASCO 17:21, 1976.
8. VOGEL CL, BAYLEY AC, BROOKER RJ, ET AL. A phase II study of adriamycin (NSC-123127) in patients with hepatocellular carcinoma from Zambia and the United States. Cancer 39:1923–1929, 1977.

Changes in Glomerular Filtration Rate Associated With High-Dose Methotrexate Therapy in Adults[1,2,3]

Brief Reports and Preliminary Communications

Stephen B. Howell* and Johanna Carmody[4,5]

Methotrexate (MTX) nephrotoxicity was a major source of morbidity and mortality in early studies of high-dose MTX with citrovorum factor rescue (1,2).[6] In patients receiving 1–15 g/m² of MTX, renal toxicity developed in 47% of treatment courses in 73% of patients (3). Vigorous hydration and alkalinization of the urine have reduced the incidence of clinical nephrotoxicity to 15% of courses and 39% of patients (4), but has not eliminated the problem altogether. A recent study in children (5) reported that even in those patients who did not go on to develop clinically evident renal toxicity, there was still a marked drop in glomerular filtration rate (GFR) following high-dose MTX. Inulin clearance fell an average of 51%, and creatinine clearance an average of 42% from pretreatment to 40 hours posttreatment. The study reported here was undertaken to determine whether major subclinical changes in GFR were occurring in adults receiving urinary alkalinization during high-dose MTX treatment who did not develop clinically apparent nephrotoxicity.

MATERIALS AND METHODS

Treatment

GFR determinations were performed in patients receiving weekly high-dose MTX with citrovorum factor rescue as part of a phase II study. Urine was alkalinized with $NaHCO_3$, 3.0 g orally every 3 hours starting 12 hours before MTX infusion and continuing for 48 hours after treatment to maintain urine pH at 7.0. During the 2 hours prior to MTX infusion, each patient received 1 liter of 5% dextrose containing 43 mEq $NaHCO_3$ iv, and the urine output was maintained at 3000 ml/day for the subsequent 48 hours. MTX was given by rapid iv infusion over 15 minutes, and citrovorum factor rescue was started 24 hours later with 10 mg/m² iv, then the same dose orally every 6 hours for 12 doses.

GFR Determination

GFR was estimated using ^{125}I-iothalamate (IOT) clearance (6). ^{125}I-IOT (Abbott Laboratories, Chicago, Ill, 0.278 millicurie/mg), 0.2 microcurie/kg, was diluted in 10 ml of normal saline and injected rapidly iv to establish a plasma level of approximately 7000 cpm/ml. This was then maintained by infusing ^{125}I-IOT, 9 microcuries/hour in 60 ml of normal saline via an IMED constant infusion pump. After a 45-minute equilibration period, a 2-hour timed urine collection was obtained. Plasma samples were taken at the beginning and end of the urine collection. The concentration of ^{125}I-IOT was determined in plasma and urine by counting 2-ml aliquots in a gamma counter. IOT clearance was calculated from the standard clearance equation using the average of the plasma IOT concentration at the beginning and end of the timed urine collection. Bladder emptying was demonstrated to be complete by iv pyelogram in all patients. The standard error (SE) of four repeated studies in a 24-hour period in the same patient was ±1.3 ml/minute/m² (3.5% of the mean). The SE of four studies repeated at weekly intervals on the same patient under conditions of equivalent hydration and urine flow was ±2.8 ml/minute/m² (6.3% of the mean).

RESULTS

GFR determinations were made in 17 patients during 20 courses of treatment; all patients had a normal serum creatinine (< 1.2 mg/dl) and creatinine clearance (> 60 ml/minute) prior to therapy. None of these patients developed clinical signs or symptoms of MTX toxicity or a significant change

[1]Received Feb 2, 1977; revised May 13, 1977; accepted May 23, 1977.

[2]Supported in part by Public Health Service grants CA-17979 and CA-06516 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3]Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-.

[4]Sidney Farber Cancer Institute, Boston, Mass.

[5]We wish to thank Dr. George Canellos for advice on conducting this study.

[6]Citrovorum factor: NSC-3590; CAS reg. No. 6055-86-5; glutamic acid, *N*-[*p*-[[(2-amino-5-formyl-5,6,7,8-tetrahydro-4-hydroxy-6-pteridinyl)methyl]amino]benzoyl]-, calcium salt.

Reprint requests to: Stephen B. Howell, MD, Sidney Farber Cancer Institute, 44 Binney St, Boston, Mass 02115.

TABLE 1.—Change in GFR determined before and 0–2 hrs after bolus MTX infusion

Dose (g/m^2)	No. of patients	Average change in GFR (% ± SE)
0.5	1	−1.6
1.0	3	−9.7±1.9
3.0	5	−15.8±6.4
7.5	4	−19.5±2.9

in serum creatinine. Serial GFR determinations were made during the 2 hours preceding treatment, and at one or more of the following times: Hours 0–2, 6–8, and 22–24 after treatment.

The change in GFR taking place during the first 2 hours after treatment was measured in 13 courses of treatment in ten patients receiving 0.5–7.5 g/m^2 of MTX. The data in table 1 demonstrate that there was a small acute fall in GFR that was proportional to dose, ranging from an insignificant drop at a dose of 0.5 g/m^2 to a drop of 19.5% at a dose of 7.5 g/m^2. In one patient, the change in GFR occurring during the first 2 hours was measured weekly for 4 weeks as the MTX dose was escalated from 0.5 to 1.0, 3.0, and 7.5 g/m^2. In this single patient, the acute decrease in GFR was very close to log-linear with the dose varying from 1.6% at 0.5 g/m^2 to 17.4% at 7.5 g/m^2 with a coefficient of variation of 0.998.

GFR was measured at multiple time points during the first 24 hours in three patients receiving 3.0 g/m^2 and in another three patients receiving 7.5 g/m^2 of MTX. Figure 1 shows that five of these six patients had a fall in GFR occurring primarily in the first 2 hours after treatment. Return of GFR to near baseline by 24 hours was documented in four of these five patients. In one patient who had a fall of 40% in GFR right after MTX infusion, GFR recovered to above baseline by 6–8 hours.

The return of GFR to near baseline was confirmed when GFR was measured prior to and at 22–24 hours after treatment in 12 patients. Averaging all patients, the GFR increased 5.2% and the serum creatinine increased 0.12 ± 0.4 mg/dl during this period, and there were no significant differences between patients receiving 1–7.5 g/m^2.

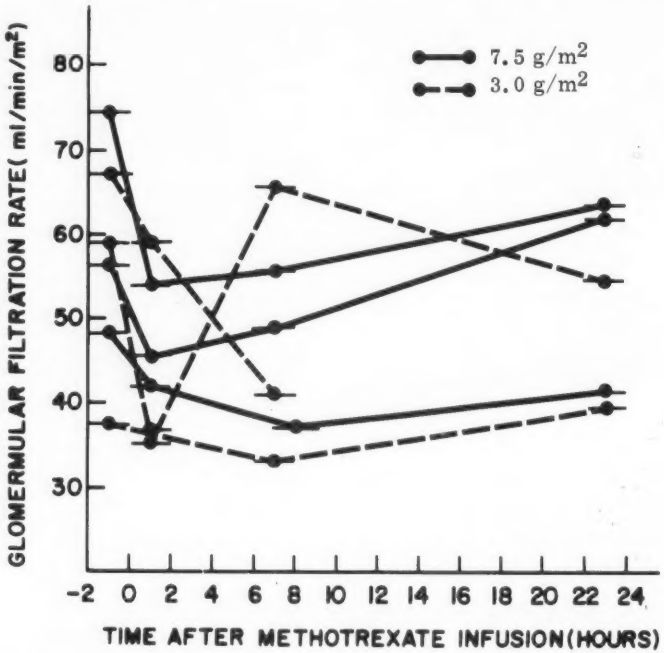


FIGURE 1.—Serial change in GFR following rapid infusion of 3.0 and 7.5 g/m^2 of MTX. Urine pH was maintained at > 7.0 and urine output was >3000 ml/day. Each point represents a 2-hr determination of GFR by the IOT clearance technique.

DISCUSSION

This study indicates that large subclinical changes in GFR are not occurring in adults receiving high-dose MTX with urinary alkalinization. Most patients had a small reversible drop in GFR, and this occurred primarily within the first 2 hours following MTX injection. The magnitude of the acute fall in GFR was proportional to the MTX dose. In some patients, GFR continued to fall during the subsequent 6 hours; however, in some, partial recovery was already evident by 6–8 hours after treatment. GFR had returned toward baseline in all patients followed with serial GFR determination up to 24 hours after MTX injection.

The mechanism by which high-dose MTX therapy causes subclinical changes in GFR is unknown. MTX may reduce GFR indirectly by reducing cardiac output through a decrease in contractility or through a fall in heart rate associated with the vagotonia of vomiting. Alternatively, MTX may affect the kidney directly, causing a change in renal blood flow, direct damage to the tubules, or the formation of precipitates in the tubular urine.

There is some evidence that this latter mechanism analogous to acute uric acid nephropathy is the cause of the more profound decrease in GFR associated with clinical nephrotoxicity: MTX is poorly soluble in acid urine (2,7) and during the first 2 hours after bolus MTX infusion when the excretion rate of MTX is very high, the urine is supersaturated and yellow crystals and amorphous tubular casts appear in the sediment (2,4). Urinary alkalinization increases the solubility of MTX, and reduces the incidence of clinical nephrotoxicity (4); it is thought to do this by preventing crystal formation (2).

The observation of relatively minor changes in GFR associated with high-dose MTX therapy in adults stands in contrast to the observations of Link et al (5) who found major changes in inulin clearance in children. The discrepancies between these two studies suggest that alkalinization alters the influence of high-dose MTX therapy on subclinical changes in GFR by whatever mechanism this occurs, since, with the exception of age, the major difference in the two study populations was the use of alkalinization in the adults.

REFERENCES

1. JAFFE N, and TRAGGIS D. Toxicity of high-dose methotrexate (NSC-740) and citrovorum factor (NSC-3590) in osteogenic sarcoma. Cancer Chemother Rep Part 3, vol 6(1):31–36, 1975.
2. STOLLER RG, JACOBS SA, DRAKE JC, ET AL. Pharmacokinetics of high-dose methotrexate (NSC-740). Cancer Chemother Rep Part 3, vol 6(1):19–24, 1975.
3. PITMAN SW, PARKER LM, TATTERSALL MHN, ET AL. Clinical trial of high-dose methotrexate (NSC-740) with citrovorum factor (NSC-3590)—toxicologic and therapeutic observations. Cancer Chemother Rep Part 3, vol 6(1):43–49, 1975.
4. PITMAN SW, and FREI E, III. Weekly methotrexate-calcium leucovorin rescue: effect of alkalinization on nephrotoxicity; pharmacokinetics in the CNS; and use in CNS non-Hodgkin's lymphoma. Cancer Treat Rep 61:695–701, 1977.
5. LINK DA, FOSBURG MT, INGELFINGER JR, ET AL. Renal toxicity of high-dose methotrexate. Pediatr Res 10:455, 1976.
6. SIGMAN EM, ELWOOD CM, and KNOX F. The measurement of glomerular filtration rate in man with sodium iothalamate 131-I (Conray). J Nucl Med 7:60–68, 1965.
7. TATTERSALL MHN, PARKER LM, PITMAN SW, ET AL. Clinical pharmacology of high-dose methotrexate (NSC-740). Cancer Chemother Rep Part 3, vol 6(1):25–29, 1975.

Effect of Hydration on Plasma-Methotrexate Levels[1,2]

J. L. Romolo,* N. H. Goldberg, K. R. Hande, and S. A. Rosenberg[3,4]

Brief Reports and Preliminary Communications

The use of vigorous iv hydration in patients receiving high-dose methotrexate (MTX) with citrovorum factor (CF)[5] rescue has been advocated as a safeguard for the prevention of renal toxicity. MTX is rapidly excreted into the urine, so that resultant urinary concentrations in the first 12 hours may exceed the 2-mM solubility of MTX in the urine of ≤ pH 5.5 (1). In order to prevent precipitation of MTX in the renal tubules, Stoller et al (1) stressed the need for urinary alkalinization and high urine volume during and after high-dose MTX treatment (1). To determine whether hydration affects the renal clearance of MTX, plasma-MTX levels have been measured in 29 treatment courses of adjuvant high-dose MTX with CF rescue with and without vigorous iv hydration in patients being treated for osteogenic sarcoma.

METHODS

Seven patients (four males and three females) with ages ranging from 10 to 47 years (mean age, 23.6 years), all post-amputation for osteogenic sarcoma of the extremity and free of metastases, were studied after an informed consent was obtained. The patients served as their own controls and received either maintenance or vigorous iv hydration on alternate MTX treatment cycles.

MTX was given by a constant gravity-drip iv infusion over 6 hours; the dose of MTX was 250 mg/kg except for one patient (No. 3) who received 100 mg/kg. MTX treatment cycles were administered every 21 days; each patient had alternating vigorous or maintenance hydration. CF rescue was initiated 2 hours after the completion of the MTX infusion. CF was given at a dose of 15 mg/m² every 6 hours iv for a total of eight doses.

Vigorous hydration consisted of the iv infusion of 200 ml/hour of D5W with 100 mEq of $NaHCO_3$/liter and 20 mEq of KCl/liter starting 12 hours prior to MTX and continuing for 48 hours after therapy. Maintenance hydration consisted of the iv infusion of 75 ml/hour of D5W with 200 mEq of $NaHCO_3$/liter[6] and 40 mEq of KCl/liter starting 12 hours prior to MTX and continuing for 36 hours after therapy.

The plasma concentration of MTX was measured by a competitive protein-binding assay using *Lactobacillus casei* dihydrofolate reductase as described by Myers et al (2). This assay is specific for MTX and permits detection of levels as low as 5×10^{-9} M. The predominant circulating folate, 5-methyl tetrahydrofolate, and the clinical rescue agent, CF, do not interfere with the assay, nor does the MTX metabolite, 4-amino-4-deoxy-10-methylpteroic acid.

During each treatment cycle, the following parameters were measured: intake and output; pH of each voided urine specimen; daily body weight; daily cbc and platelet counts, electrolytes, BUN, and creatinine; 24-hour urine creatinine clearance; liver function tests; and plasma-MTX levels at 6, 12, 24, 48, and 72 hours following the initiation of the MTX infusion.

RESULTS

Seven patients were studied for a total of 29 MTX–CF administration cycles; there were 13 cycles with maintenance and 16 with vigorous hydration. The results in these seven patients are shown in figure 1 and table 1. During maintenance hydration, the mean total fluid intake was 1901 ± 140 ml/day (mean ± 1 SE) with a mean total fluid output of 1752 ± 166 ml/day. When this same patient population was treated with vigorous hydration, the mean total fluid intake was 4124 ± 234 ml/day and the mean total fluid output was 3516 ± 266 ml/day.

[1]Received Feb 7, 1977; revised June 1, 1977; accepted June 8, 1977.

[2]Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-.

[3]Division of Cancer Treatment (DCT), National Cancer Institute (NCI), Bethesda, Md.

[4]We wish to thank Ada Brooks for performing the determinations of plasma-methotrexate levels.

[5]Citrovorum factor: NSC-3590; CAS reg. No. 6055-86-5; glutamic acid, *N*-[*p*-[[(2-amino-5-formyl-5,6,7,8-tetrahydro-4-hydroxy-6-pteridinyl)methyl]amino]benzoyl]-, calcium salt.

**Reprint requests to:* J. L. Romolo, MD, DCT, NCI, Bldg 10, Rm 10N116, National Institutes of Health, Bethesda, Md 20014.

[6]The amount of $NaHCO_3$ administered was increased as necessary to maintain a urine pH of ≥ 7.

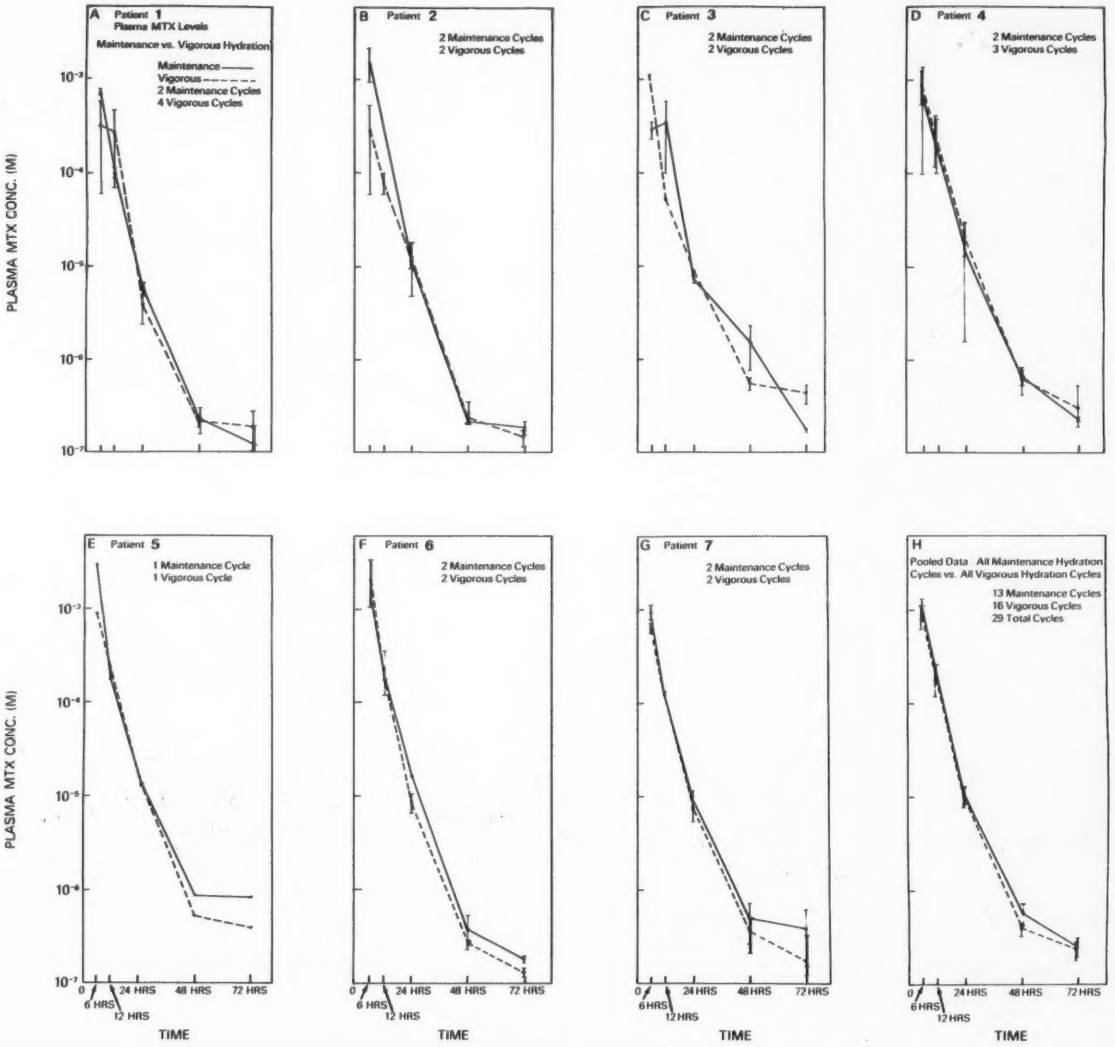


FIGURE 1.—Effect of hydration on plasma-MTX levels.

FIGURE 1.—Effect of hydration on plasma-MTX levels.

TABLE 1.—Effect of hydration on plasma-MTX levels*†

Patient No.	Patient age/sex	Type of hydration	No. of cycles	Average intake, ml/24 hrs (SEM)	Average output, ml/24 hrs (SEM)	Degree and duration of emesis, ml/24 hrs	Average weight change (kg)	Average BUN (mg/100 ml)	Average 24-hr creatinine clearance (ml/min)	Average wbc count (cells/mm³)	Additional CF	24-hr MTX level (SEM)	48-hr MTX level (SEM)
1	16/M	Maintenance	2	1809 (399)	2109 (513)	970 (2-day average)	−2.3	8.2	118	6150	None	5.7×10^{-6} (0.9)	2.3×10^{-7} (0.7)
		Vigorous	4	3734 (474)	3369 (527)	954 (1.5-day average)	−1.2	6.7	144	7425	None	3.9×10^{-6} (1.5)	2.2×10^{-7} (0.3)
2	19/F	Maintenance	2	1902 (386)	1675 (453)	825 (2-day average)	−1.3	6.3	85	3850	None	10.5×10^{-6} (5.7)	2.4×10^{-7} (1.2)
		Vigorous	2	4247 (969)	3336 (936)	875 (2-day average)	−0.1	4.5	74	4000	None	10.2×10^{-6} (0.6)	2.4×10^{-7} (0.3)
3	47/F	Maintenance	2	1904 (313)	1448 (407)	280 (1-day average)	+0.6	8.9	65	5300	None	7.2×10^{-6} (0.6)	15.2×10^{-7} (7.6)
		Vigorous	2	3828 (741)	3768 (712)	452 (1.5-day average)	−0.2	6.5	117	6000	1 ×	9.0×10^{-6} (—)	5.5×10^{-7} (0.8)
4	40/M	Maintenance	2	1727 (327)	1826 (383)	705 (3.5-day average)	−4.5	10.5	86	5650	1 ×	15.3×10^{-6} (13.7)	6.6×10^{-7} (1.2)
		Vigorous	3	4380 (423)	3811 (576)	1056 (2.7-day average)	−1.7	8.3	101	6033	2 ×	21.3×10^{-6} (8.3)	6.3×10^{-7} (2.0)
5	10/M	Maintenance	1	1842 (603)	1604 (539)	82 (2 days)	−2.0	10.3	75	8600	1 ×	16.8×10^{-6} (—)	8.4×10^{-7} (—)
		Vigorous	1	4137 (975)	4015 (1595)	375 (1 day)	−1.7	7.0	123	7100	None	17.0×10^{-6} (—)	5.1×10^{-7} (—)
6	18/M	Maintenance	2	2404 (507)	1393 (380)	? volume (0.5-day average)	−1.9	9.3	133	8050	None	16.5×10^{-6} (—)	3.8×10^{-7} (1.5)
		Vigorous	2	4329 (856)	2951 (691)	583 (2-day average)	−1.7	6.3	131	6900	None	8.6×10^{-6} (1.9)	2.7×10^{-7} (0.1)
7	15/F	Maintenance	2	1809 (306)	2061 (517)	560 (2.5-day average)	−1.9	8.5	110	7250	1 ×	9.3×10^{-6} (2.1)	4.9×10^{-7} (2.2)
		Vigorous	2	4440 (435)	3483 (725)	900 (1.5-day average)	−1.0	4.9	127	6150	None	7.6×10^{-6} (2.3)	3.5×10^{-7} (1.4)
All patients		Maintenance		1901 (140)	1752 (166)							Mean, 1.08×10^{-5} (0.22)	Mean, 6.03×10^{-7} (1.56)
		Vigorous		4124 (234)	3516 (266)							Mean, 1.05×10^{-5} (0.22)	Mean, 3.97×10^{-7} (0.56)

*Total of 29 cycles (13 maintenance and 16 vigorous).
†All patients had urine pH ≥ 7.

The degree of hydration did not affect the elimination of MTX from the systemic circulation of any patient (fig 1). The disappearance of MTX from the circulation was similar whether iv fluids were given at a rate of 75 or 200 ml/hour. For all patients the mean plasma-MTX level at 6 hours was $1.07 \pm 0.24 \times 10^{-3}$ M with maintenance hydration and $0.85 \pm 0.24 \times 10^{-3}$ M with vigorous hydration; the mean plasma-MTX level at 24 hours was $1.08 \pm 0.22 \times 10^{-5}$ M with maintenance hydration and $1.05 \pm 0.22 \times 10^{-5}$ M with vigorous hydration ($P < 0.475$); corresponding levels at 48 hours were 6.03 ± 1.56 and $3.97 \pm 0.56 \times 10^{-7}$ M respectively ($P < 0.15$).

Patients were given additional CF if the 48-hour plasma-MTX level was $> 4 \times 10^{-7}$ M. There was no correlation between the need for additional CF and the volume of fluids administered. Additional CF was required three times after maintenance hydration and three times after vigorous hydration.

The degree of hydration had no effect on the amount or duration of emesis. On maintenance hydration, emesis lasted a mean of 1.9 days and averaged 570 ml/day compared to a mean of 1.7 days and an average of 742 ml/day when vigorous hydration was used. Patients receiving maintenance hydration experienced a mean weight loss of 1.9 kg/treatment cycle whereas patients receiving vigorous hydration experienced a mean weight loss of 1.1 kg/treatment cycle ($P < 0.15$).

No patient, regardless of volume or rate of fluid administration, exhibited sustained myelosuppression, as measured by wbc or platelet counts at weekly intervals. No patient experienced impairment of renal function, as measured by elevation of BUN or decrease in 24-hour urine creatinine clearance performed 3 weeks after each cycle. No patient experienced compromise of hepatic function, as measured by monthly liver function tests (SGOT, SGPT, alkaline phosphate, and total/direct bilirubin).

DISCUSSION

The effect of the volume and rate of iv fluid replacement on the disappearance of MTX from the systemic circulation was studied in seven patients with osteogenic sarcoma. No difference was noted in plasma-MTX levels at various times following the infusion of 250 mg/kg of MTX in patients receiving iv fluids at the rate of 75 ml/hour for 48 hours compared to those receiving 200 ml/hour for 60 hours. Myelosuppression, impairment of renal function, or compromise of hepatic function was not seen in any patient, regardless of the amount of iv fluids administered when urinary pH was kept > 7.0. It thus appears that the utilization of vigorous hydration, designed to prevent toxicity during the administration of high-dose MTX, does not lead to more rapid clearance of MTX or subsequent decreased therapeutic effectiveness. Vigorous hydration should continue to be utilized in high-dose MTX therapy because it does not appear to alter MTX pharmacokinetics.

REFERENCES

1. STOLLER RG, JACOBS SA, DRAKE JC, ET AL. Pharmacokinetics of high-dose methotrexate (NSC-740). Cancer Chemother Rep Part 3, vol 6(1):19–24, 1975.
2. MYERS C, LIPPMAN M, ELIOT H, ET AL. Competitive protein binding assay for methotrexate. Proc Natl Acad Sci USA 72:3683–3686, 1975.

Treatment of Refractory Acute Leukemia With High-Dose Chemotherapy [1]

Brief Reports and Preliminary Communications

B. S. Kramer,* F. R. Appelbaum, G. P. Herzig,[2] R. G. Graw, Jr,[3] and A. S. Levine [4]

TEAM Chemotherapy[5]

During the past decade, there has been a marked improvement in remission induction rate and duration in both children and adults with acute lymphoblastic leukemia (ALL) (1). However, a significant number of patients with this disease experience relapse and rapidly develop resistance to conventional chemotherapy. Evidence from work with animal tumor models demonstrates that higher doses of some antineoplastic agents, especially alkylating agents, have correspondingly greater antitumor effects (2). Thus, the question arises whether very high-dose chemotherapy can induce a significant percentage of remissions in patients with ALL or acute myelocytic leukemia (AML) who are resistant to conventional chemotherapy. In this study, we have attempted to treat such patients with very high-dose combination chemotherapy. The regimen chosen was similar to one we have employed as a preparatory regimen for allogeneic bone marrow transplantation in leukemia (3). The agents were chosen to avoid overlapping toxic effects other than myelosuppression. Methyl-CCNU was chosen because of its ability to cross the blood-brain barrier, and because of its delayed hematopoietic toxicity relative to other agents.

[1]Received Feb 7, 1977; revised June 7, 1977; accepted June 20, 1977.

[2]*Present address:* Department of Medicine, Washington University School of Medicine, St. Louis, Mo.

[3]*Present address:* 2772 Rutland Rd, Davidsonville, Md.

[4]Division of Cancer Treatment (DCT), National Cancer Institute (NCI), Bethesda, Md.

[5]TEAM = 6-thioguanine, cyclophosphamide (Endoxan), cytosine arabinoside, and methyl-CCNU.

6-Thioguanine: NSC-752; CAS reg. No. 1832–72–0; purine-6-thiol, 2-amino-.

Cyclophosphamide: NSC-26271; CAS reg. No. 6055–19–2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

Cytosine arabinoside: NSC-63878; CAS reg. No. 69–74–9; cytosine, 1-β-D-arabinofuranosyl-; monohydrochloride.

Methyl-CCNU: NSC-95441; CAS reg. No. 33073–59–5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-.

**Reprint requests to:* Barnett S. Kramer, MD, DCT, NCI, Bldg 10, Rm 2B50, National Institutes of Health, Bethesda, Md 20014.

PATIENTS AND METHODS

Seventeen patients, 10 males and seven females, were treated in the Pediatric Oncology Branch of the NCI with high-dose combination chemotherapy, TEAM (*see* table 1). Twelve patients had ALL and the remainder had AML as established by morphologic examination of bone marrow aspirates. The patients' ages ranged from 6 to 25 years, the duration of disease ranged from 5 to 89 months (median, 22 months), and the number of prior relapses ranged from none to eight.

All patients had had prior exposure to conventional agents including prednisone, vincristine, methotrexate, 6-mercaptopurine, daunorubicin, and L-asparaginase. All but one patient also had had prior exposure to one or more of the agents in TEAM. For the first four patients studied, cyclophosphamide was administered only on Days 1 and 2. When this was found to be tolerable, cyclophosphamide was increased to 3 days as shown in table 1.

Bone marrow aspirations were done every 2 weeks during therapy, and every 4 weeks after remission was documented. Length of remission was computed from the day M1 bone marrow was achieved (< 5% blast cells with normal cellularity) to the day that the bone marrow showed > 5% blast cells.

RESULTS

Of the 17 patients treated, three attained complete remissions (CRs) lasting 73, 59, and 42 days. All three had ALL. The third of these three patients was given maintenance therapy but his remission was the shortest. In each case, relapse occurred in the bone marrow at a time when the central nervous system was uninvolved.

Of the remaining 14 patients, one died of *Candida* sepsis before marrow recovery and all others were found to have recurrent leukemia documented either by premortem bone marrow examination or at autopsy. Four patients received a second course

TABLE 1.—TEAM induction regimen

Drug	Dose and schedule	Day 1	2	3	4	5	6
Cyclophosphamide	50 mg/kg/day, iv (2-hr infusion)	×	×				×
Cytosine arabinoside	100 mg/m² q12hrs, iv by rapid infusion			×	×	×	×
6-Thioguanine	100 mg/m² q12hrs, iv by rapid infusion			×	×	×	×
Methyl-CCNU	150 mg/m², orally					×	

of TEAM; two subsequently died of infection without marrow recovery and two died with residual marrow disease. One patient who failed to respond to one course of TEAM achieved a CR when subsequently treated with L-asparaginase, vincristine, daunorubicin, and prednisone. Of 15 evaluable patients, three developed marrow hypoplasia but blast cells were never completely eliminated; blast cells were not identified on at least one marrow examination in each of the remaining 12 patients. Of the three evaluable patients with AML, blast cells were never entirely eliminated in two; the third had marrow aplasia but subsequently the marrow was repopulated with blast cells. Bone marrow examination was not performed in two patients until subsequent relapse.

Toxicity

Medullary.—Severe marrow hypoplasia was universal, with some patients exhibiting no hematopoietic elements on marrow biopsy for up to 3 weeks after initiation of therapy. This was reflected by severe granulocytopenia. The mean number of days spent with peripheral granulocytes < 500/mm³ was 29.3 (median, 23 days; range, 9–93 days). Febrile episodes occurred with every course of treatment, and seven patients had documented sepsis.

Extramedullary.—Anorexia, nausea, and vomiting were common. Eight patients developed hematuria. One case of severe exfoliative dermatitis occurred. There were transient abnormalities in liver function tests in nine courses, but no clinical evidence of hepatic failure.

DISCUSSION

In this study, we attempted to induce remission in patients with acute leukemia who were resistant to conventional chemotherapy. We used a chemotherapy regimen similar to one which has been used prior to allogeneic bone marrow transplantation. Toxicity was severe and results were disappointing.

There are few data in the literature with which to compare our results. Steuber et al (4), using cyclophosphamide at 40 mg/kg/day on 4 successive days, reported seven responses in 16 children with advanced leukemia. However, only one of these seven responders had a CR as defined by an M1 marrow with normal cellularity. Even that patient was felt to have inadequate peripheral blood cell counts to tolerate maintenance therapy. Bryan et al (5), using a combination of cytosine arabinoside (100 mg/m²) and 6-thioguanine (100 mg/m²) every 12 hours for 6–31 days, achieved CRs in nine of 19 patients. As with our therapy, granulocytopenia was severe, and remission duration was short (median, 28 days). Wang et al (6), using cytosine arabinoside in doses of 150–200 mg/m²/day for 5 days, observed only three CRs in 46 children with refractory acute leukemia.

In conclusion, we find little to support the combined use of very high doses of cyclophosphamide, methyl-CCNU, cytosine arabinoside, and 6-thioguanine as a re-induction regimen in refractory acute leukemia.

REFERENCES

1. GUNZ F, and BAIKIE AG. Leukemia, 3rd ed. New York, Grune and Stratton, 1974, pp 555–560.
2. BRUCE WR, MEEKER BE, and VALERIOTE FA. Comparison of the sensitivity of normal hematopoietic and transplanted lymphoma colony-forming cells to chemotherapeutic agents administered in vivo. J Natl Cancer Inst 37:233–245, 1966.
3. HERZIG GP, BULL MI, DECTER J, ET AL. Bone marrow transplantation in leukemia and aplastic anemia: NCI experience with four grafting regimens. Transplant Proc 7:817–821, 1975.
4. STEUBER CP, FERNBACH DJ, STARLING KA, ET AL. High-dose cyclophosphamide (NSC-26271) therapy in childhood leukemia. Cancer Chemother Rep 58:417–419, 1974.
5. BRYAN JH, HENDERSON ES, and LEVENTHAL BG. Cytosine arabinoside and 6-thioguanine in refractory acute lymphocytic leukemia. Cancer 33:539–544, 1974.
6. WANG JJ, SELAWRY OS, VIETTI TJ, ET AL. Prolonged infusion of arabinosyl cytosine in childhood leukemia. Cancer 25:1–6, 1970.

Heparin and Polychemotherapy for Treatment of Lung Cancer [1,2]

Brief Reports and Preliminary Communications

Jay J. Rohwedder[3,*] and Edmundo Sagastume [4]

Interest in anticoagulation for treatment of solid tumors was stimulated by the recognition that: (*a*) heparin, coumarins, fibrinolysins, and antifibrinogen agents which inhibit coagulation reduce the incidence of metastases in some, *but not all*, experimental tumor systems; and (*b*) the deposition of a fibrin matrix may be a prerequisite for vascularization and growth of *some* primary tumors. This information is summarized in two recent publications (1,2).

Clinical studies of anticoagulation as an adjuvant to chemotherapy for lung carcinoma have also produced disparate conclusions (3,4). Elias et al (3) obtained very encouraging preliminary results: hyperfibrinogenemic patients with metastatic lung carcinoma responded to a single course of combination chemotherapy with cyclophosphamide, 5-fluorouracil (5-FU), 6-thioguanine, methotrexate, and vincristine[5] while anticoagulated with heparin. Massive tumor regression, > 50% in one study, was demonstrated in 15 of 25 patients treated (3).

In contrast, the three other clinical studies of anticoagulation for lung cancer suggested little reason for optimism (4–6). The results in the most recent clinical study clearly were negative: only one of 19 patients (5%) treated by the Veterans' Administration Lung Cancer Study Group (VALCSG) with iv cyclophosphamide plus heparin showed tumor regression (4). The patients' 16-week median survival after treatment was about that expected with cyclophosphamide alone.

In the present study, the VALCSG evaluated the efficacy of heparin plus cyclophosphamide, 5-FU, 6-thioguanine, methotrexate, and vincristine, the combination Elias et al (3) found useful.

PATIENTS AND METHODS

Patients with histologically proven, unresectable, measurable, or evaluable lung cancer unsuitable for radiation therapy were considered for study if their Karnofsky performance score was ≥ 40 (40 = disabled: requires special care and assistance). A positive brain scan or a history of peptic ulcer disease, gastrointestinal (GI) hemorrhage, bleeding diathesis, or hemoptysis was reason for exclusion from heparin therapy. Sixteen men were entered in the study, nine with evaluable and seven with measurable disease (table 1). Eight men had evidence of extrathoracic metastases; nevertheless, the Karnofsky performance score was quite favorable in all of the patients. The median age was 57 years.

Aqueous heparin was administered by iv infusion using Harvard constant-infusion syringe pumps. The poor reproducibility and failure of the whole-blood clotting time to detect levels of heparin in excess of the anticoagulant range were avoided by monitoring heparin by a modification of the thrombin clotting time (TCT) designed to be sensitive to heparin in the anticoagulant range (7). Dosage was increased until TCTs between 16 and 45 seconds (baseline, 7–9 seconds) indicated the presence of 0.2–0.4 heparin antithrombin units/ml plasma. This concentration encompasses the anticoagulant range and produces a Lee White whole-blood clotting time and partial thromboplastin time which are two to three times normal (7). Anticoagulation was discontinued the day after the last chemotherapy. No anticoagulation was used between courses of chemotherapy.

Chemotherapy was given every 4 weeks after 48 hours of stable anticoagulation: cyclophosphamide was given on Day 1 (600 mg iv); 5-FU was given on Days 2–4 (12 mg/kg iv); methotrexate and 6-thioguanine were given on Days 2–8 (5 mg and 1 mg/kg

[1]Received Feb 16, 1977; revised Apr 4, 1977; accepted Apr 20, 1977.

[2]This study was conducted by the Veterans' Administration (VA) Lung Cancer Study Group.

[3]Department of Internal Medicine and Pulmonary Service, Ann Arbor VA Hospital, Mich.

[4]Pulmonary Division, Department of Internal Medicine, University of Michigan Hospital, Ann Arbor.

[5]Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8.

6-Thioguanine: NSC-752; CAS reg. No. 1832-72-0; purine-6-thiol, 2-amino-.

Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-.

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1).

**Reprint requests to:* Jay J. Rohwedder, MD, VA Hospital, 2215 Fuller Rd, Ann Arbor, Mich 48105.

TABLE 1.—Treatment and results

Patient No.	Cell type	Prior therapy	Performance score	No. of treatment courses	Tumor response*†
1	Large cell carcinoma	None	70	3	PR
2	Adenocarcinoma	Irradiation	90	2	NR
3	Adenocarcinoma	None	70	1	NR
4	Squamous cell carcinoma	None	60	2	NR
5	Squamous cell carcinoma	None	60	1	NR
6	Squamous cell carcinoma	None	70	3	NR
7	Squamous cell carcinoma	Irradiation	50	2	NR
8	Squamous cell carcinoma	None	80	1	NR
9	Squamous cell carcinoma	None	50	1	NR
10	Squamous cell carcinoma	Irradiation	70	1	NR
11	Squamous cell carcinoma	None	70	5	PR
12	Adenocarcinoma	None	90	6	NR
13	Squamous cell carcinoma	None	90	7	PR
14	Adenocarcinoma	None	70	2	NR
15	Squamous cell carcinoma	None	70	2	NR
16	Large cell carcinoma	None	70	2	NR

*PR = partial response: all $< 1/3$ reduction in tumor diameter, without change in performance score. NR = no response.
† Median survival was 12 wks.

orally respectively); and vincristine was given on Day 8 (0.05 mg/kg iv). TCTs were obtained daily while heparin was given. Cbc counts and 12 automated blood chemistries were done twice weekly. Each patient's chest roentgenograms and Karnofsky performance scores were evaluated monthly.

RESULTS

Each of the 16 patients begun on the study completed at least one course of anticoagulation and chemotherapy (table 1). The major limiting toxicity was bone marrow depression. Leukopenia (wbc count 1000–2000/mm^3) and/or thrombocytopenia (platelet count 1500–91,000/mm^3) occurred within 4 days of the completion of a treatment course in one half of the patients. Therapy was believed to be a contributing cause in two of the three patients who developed severe infections while leukopenic after completing the first treatment course. Three of the five patients in whom occult GI blood loss was recognized required transfusion because bleeding made pre-existing chronic anemia worse. Therapy was stopped in two patients because of bleeding: one had repeated occult GI blood loss and one had hemoptysis. Severe stomatitis, total alopecia, and nausea were common. No renal or hepatic toxicity was identified. When all the toxicities were considered, it was felt that nine of the 16 patients tolerated the regimen well.

Pretreatment thrombocytosis and elevated serum fibrinogen were present in 14 patients (88%). All of the patients were resistant to heparin and required larger doses than is usual in patients with non-neoplastic disease. Chemotherapy neither affected the dose of heparin required nor caused a consistent rise or fall in the serum fibrinogen. Posttreatment fibrinogen levels were higher than pretreatment values in six patients (38%).

A decrease in tumor diameter was seen after the first course of treatment in three patients but in each the reduction was less than one third. All of the patients are now dead. The median survival measured from the first day of chemotherapy was 12 weeks, with the range from 4 days to 43 weeks. In no patient was treatment followed by improvement in the Karnofsky performance score. Autopsies were done on 11 patients (69%). There was no evidence that anticoagulation had favorably or unfavorably influenced the extent of tumor necrosis or the number of metastases.

DISCUSSION

O'Meara and Jackson's (8) observation that certain tumors deposit fibrin at their periphery resulted in conjecture as to whether cancers might thereby acquire a lattice for growth, a coating to avoid immunosurveillance, and a shield to deflect chemotherapy, all reasons to attempt clinical trials with fibrinolysin or anticoagulant treatment (1,2). Additional support for anticoagulants seemed to be found in animal experiments in which tumor cells were surrounded by thrombi before endothelial permeation. Furthermore, anticoagulants exhibited modest but variable cytotoxic activity and reduced pulmonary metastases in some (allogenic) tumor-host systems (1,2).

Unhappily, in different (syngeneic) tumor-host experiments, heparin: (*a*) promoted transcapillary passage of tumor cells and prolonged their circulation; (*b*) increased the incidence of extrapulmonary metastases while decreasing spread to the lung; and (*c*) increased pulmonary metastases after long-term anticoagulation (2).

In this study, heparin plus combined chemotherapy was not shown to be superior to less toxic monochemotherapy; moreover, the patients' 12-week median survival was similar to that of patients in other VA study groups treated with cyclophosphamide alone. The failure of the regimen was conspicuous because the patients' initial Karnofsky performance scores were good. These findings are similar to those described by Thornes (6) and Edlis et al (4): there is no benefit to lung cancer patients from the anticoagulation of warfarin and/or heparin. Despite close monitoring, severe infection and troublesome occult bleeding were unacceptably frequent.

These results differ from those of Elias et al and do not encourage extended clinical cooperative trials with anticoagulation for lung cancer.

REFERENCES

1. Anticoagulation in the treatment of cancer. Symposium organized by the Division of Hematology and Medical Oncology at Hahnemann Medical College, Philadelphia, Pa. J Med (Basel) 5:1–148, 1974.
2. HAGMAR B. Experimental tumor metastases and blood coagulability. Acta Pathol Microbiol Scand (suppl)211:5–38, 1970.
3. ELIAS EG, SHUKLA SK, and MINK IB. Heparin and chemotherapy in the management of inoperable lung carcinoma. Cancer 36:129–136, 1975.
4. EDLIS HE, GOUDSMIT A, BRINDLEY C, ET AL. A trial of heparin and cyclophosphamide in the treatment of lung cancer. Cancer Treat Rep 60:575–578, 1976.
5. CLIFFTON EE, and GROSSI CE. The rationale of anticoagulants in treatment of cancer. J Med (Basel) 5:107–113, 1974.
6. THORNES RD. Oral anticoagulant therapy of human cancer. J Med (Basel) 5:83–91, 1974.
7. PENNER JA, and HISS RG. Heparin therapy in the 70's. Mich Med Center J 40:57–61, 1974.
8. O'MEARA RA, and JACKSON RD. Cytological observations on carcinoma. Ir J Med Sci 391:327–328, 1958.

Continuous Pelvic Arterial Infusion With Bleomycin for Squamous Carcinoma of the Cervix Recurrent After Irradiation Therapy [1,2,3,4]

Brief Reports and Preliminary Communications

C. Paul Morrow,* Philip J. DiSaia, Charles F. Mangan, and Leo D. Lagasse

Invasive squamous cell carcinoma of the cervix, the second most frequent malignancy of the female genital tract, is responsible for approximately 8000 deaths annually in the US.[5] Persistence or recurrence within the pelvic field of radiation is common and is often associated with intractable pain, bleeding, foul discharge, and fistulae. Palliation has been difficult to achieve in these patients by systemic chemotherapy. This is due, at least in part, to poor vascularity of the irradiated tissues which limits the access of cytotoxic agents to these tissues. Regional arterial infusion has been utilized to increase the local concentration of drug but responses have been few and of short duration (1). A new antitumor antibiotic, bleomycin (2), with significant activity against squamous cancers, has not been tested for this purpose. In this report, the results of a cooperative study employing bleomycin in pelvic arterial infusion for the palliation of recurrent squamous carcinoma of the cervix are presented.

MATERIALS AND METHODS

From November 1971 until June 1974, 20 patients from five institutions were entered in this bleomycin arterial infusion study. Criteria for admission were biopsy-proven squamous carcinoma of the cervix recurrent in the pelvis after radiation therapy, unresectability, and a life expectancy of > 8 weeks. Studies that were required prior to treatment included: (*a*) physical examination with recording of tumor distribution and size; (*b*) histologic diagnosis of recurrence; (*c*) chest roentgenogram; (*d*) intravenous pyelogram; and (*e*) hemoglobin, hematocrit, wbc and platelet counts, BUN, serum bilirubin, alkaline phosphatase, SGOT, and prothrombin time. The blood studies and tumor response were recorded weekly during treatment. After completion of the infusion, patients were examined at 3-month intervals until death.

The bleomycin infusion was carried out through a femoral arterial catheter introduced percutaneously and threaded into the lower aorta to a position between the inferior mesenteric artery and aortic bifurcation. Alternatively, bilateral hypogastric artery catheters were inserted at the time of exploratory surgery. Continuous infusion with bleomycin (20 mg/m^2/week) for a maximum of 10 weeks or a total cumulative dose of 300 mg was carried out by means of low-flow portable infusion pump. Infusion was discontinued if evidence of pulmonary toxicity appeared. Response criteria for the study were: *complete response*—disappearance of all evidence of disease for ≥ 1 month; *partial response*—≥ 50% reduction in the product of the two largest perpendicular diameters of all measurable lesions; *progressive disease*—≥ 50% increase in the two largest perpendicular diameters of any measurable lesion, or the appearance of new lesions; and *stable disease*—any response not meeting the preceding criteria. For this study, survival is the observed lifespan from the start of treatment to death or the date of last contact.

The average age of patients in the study group was 54 years with a range of 28–76 years. The original stages (FIGO) of disease were: stage I, four patients; stage II, eight patients; stage III, five patients; and unknown stage, three patients. Recurrence time following primary therapy ranged from 4 months to 19 years. Seven patients were < 1 year post-therapy and six patients were > 5 years post-therapy.

[1]Received Feb 17, 1977; accepted Apr 20, 1977.

[2]Supported by Public Health Service grants CA-19189, CA-13641, CA-15977, CA-13630, CA-12481, and CA-13632 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3]This study was conducted by the Gynecologic Oncology Group. The following institutions participated in the investigation: University of Southern California (Drs. Morrow and DiSaia), University of Pennsylvania (Dr. Mangan), University of California at Los Angeles (Dr. Lagasse), Rhode Island Hospital, and Emory University.

[4]Bleomycin: NSC-125066; CAS reg. No. 11056-06-7; [2,4'-bithiazole]-4-carboxylic acid, 2'-(2-aminoethyl)-, monohydrate.

[5]Cancer Facts and Figures. American Cancer Society, 1976.

*Reprint requests to: C. Paul Morrow, MD, Gynecologic Oncology Group Headquarters, PO Box 60, Philadelphia, Pa 19105.

RESULTS

Toxicity

Of the 20 study patients entered, ten had toxicity of moderate to severe degree (table 1). One of four patients with pulmonary toxicity died. She received 300 mg of bleomycin during a 9-week infusion period. Two patient deaths were possibly related to treatment. One of these patients died of progressive renal failure after 3 weeks of treatment. The other died of sepsis resulting from an enterovaginal fistula which appeared during therapy. Only one serious complication directly attributable to the intra-arterial catheters was reported. A patient with a retrograde femoral catheter developed an infection at the site of catheter placement which resulted in septic embolization of the distal extremity. Resolution followed removal of the catheter and antibiotic therapy. Severe stomatitis and skin blisters developed in two patients, making it necessary to discontinue therapy. One patient received 252 mg and the other 270 mg of bleomycin. The pyrogenic response characteristic of bleomycin bolus therapy was not reported in this study group.

TABLE 1.—Treatment complications

Complication	No. of occurrences (10 patients)
Myelosuppression	0
Alopecia	1
Septic emboli (arterial catheter)	1
Vaginal hemorrhage	1
Mucocutaneous ulceration	2
Enterovaginal fistulae	3
Pulmonary fibrosis, pneumonitis	4
Renal failure	4
Total	16

Response

Three patients received the bleomycin contrary to protocol stipulations and are considered unevaluable for this study, although none of the three responded. Another patient dying of renal failure during the fourth week of treatment is also unevaluable for treatment response. Among the 16 evaluable patients there were no complete responses and two partial responses, both showing slightly > 50% tumor reduction. Nine patients had stable disease during treatment, four patients had progression, and one patient had < 50% reduction. On the average, each patient received 221 mg of bleomycin (range, 80–320 mg) given during an 8.2-week infusion time (range, 8–15 weeks). All patients died from disease. The mean survival time was 7 months with a range from 1 to 19 months. The two patients exhibiting partial tumor responses survived 5 and 8 months.

DISCUSSION

Bleomycin is a mixture of sulfur-containing polypeptide antibiotics obtained in Japan from a strain of *Streptomyces verticillus*. Its cytotoxic effect results from inhibition of DNA synthesis and scission of single-stranded DNA.[6] Cell death may result from exposure to bleomycin in all phases of the cell cycle but the cell is more vulnerable in M and G_2 (3). It is an unusual drug because it is concentrated (or poorly inactivated) in squamous epithelium and has almost no myelosuppressive effect in clinical doses (4). Although bleomycin has been administered most often on a twice weekly schedule, Drewinko et al (5) increased the fractional kill of human lymphoma cells in tissue culture by prolonging treatment exposure time. Since bleomycin has a serum half-life of < 1 hour (4), a schedule which prolongs tissue levels might be expected to increase tumor response. Furthermore, squamous carcinoma has a long doubling time and a small growth fraction. Prolonged treatment will keep the nonsynchronized tumor cells exposed to drug permitting a higher fraction of proliferating cells to pass through a vulnerable phase of the generation cycle. During this time resting stem cells should be recruited into the proliferating cell pool.

Unfortunately, as this study demonstrates, all the theoretic advantages of this treatment method proved to be of no clinical importance. The poor rate of objective tumor regression and the mean survival time of 7 months do not justify the expense and effort involved. In addition, the risks have proved to be substantial. At least one patient died of treatment complications (pulmonary fibrosis) and an additional two patients died of complications that might be treatment related (sepsis, renal failure). It is our clinical impression that bleomycin can accelerate renal failure in the presence of bilateral ureteral obstruction although the BUN and serum creatinine levels at the initiation of therapy are normal. For this reason the four cases of progressive renal failure are included in the list of treatment complications. Certainly bleomycin has the potential for nephrotoxicity (6). Response is very difficult to evaluate for tumor within the field

[6]Rosenbaum C, and Carter SK. Clinical brochure: bleomycin (NSC 125066, "Bleo," BLM). Prepared by the NCI, Bethesda, Md, Mar 1970.

of prior irradiation, but in no case in this study did all clinical evidence of tumor disappear, and in only two instances was there a measurable partial response to bleomycin-infusion therapy.

The results of this study demonstrate that continuous arterial infusion of bleomycin is not useful in the management of squamous carcinoma of the cervix recurrent in the pelvis after radiation therapy.

REFERENCES

1. BATEMAN JC. Can chemotherapeutic perfusion of the pelvic viscera palliate patients with advanced or recurrent cervical cancer? JAMA 194:163, 1965.
2. BLUM RH, CARTER SK, and AGRE K. A clinical review of bleomycin—a new antineoplastic agent. Cancer 31:903, 1973.
3. BARRANCO SC, and HUMPHREY RM. The effects of bleomycin on survival and cell progression in Chinese hamster cells in vitro. Cancer Res 31:1218, 1971.
4. OHNUMA T, HOLLAND JF, MASUDA H, ET AL. Microbiological assay of bleomycin: inactivation, tissue distribution and clearance. Cancer 33:1230, 1974.
5. DREWINKO B, NOVAK JK, and BARRANCO SC. The response of human lymphoma cells in vitro to bleomycin and 1,3-bis(2-chloroethyl)-1-nitrosourea. Cancer Res 32:1260, 1972.
6. SCHAEPPI U, THOMPSON GR, FLEISCHMAN RW, ET AL. Preclinical toxicologic evaluation of bleomycin (NSC-125066) in rhesus monkeys. Cancer Chemother Rep Part 3, vol 4(1):31–39, 1973.

Serum Displacing Agents in Cancer Chemotherapy [1]

Fred J. Schiffman, Joyce M. Fisher, and Marco Rabinovitz [2,*]

Brief Reports and Preliminary Communications

Centrophenoxine, a psycho-active drug, or its active moiety, *p*-chlorophenoxyacetic acid (CPA), has been shown to potentiate the chemotherapeutic activity of several alkylating agents (1–4).[3] Clofibrate, a drug effective in lowering the concentration of blood lipids and whose active moiety is *p*-chlorophenoxyisobutyric acid (CPB), has also been reported to be an effective potentiator (5).[4] The mode of action of CPB in controlling lipid metabolism may be related to its ability to displace free fatty acids bound to serum albumin (6,7).

Chlorambucil[5] is an aromatic nitrogen mustard with a free fatty acid carrier having structural similarity to both CPA and CPB (fig 1). Since chlorambucil may have its activity limited because of its tight binding to serum albumin (8), it seemed of interest to investigate the effect of CPA and CPB on both its toxicity and binding to serum components in a tissue culture system.

MATERIALS

The sodium salt of CPA was obtained from Pfaltz and Bauer, Inc, and the sodium salt of CPB was a gift from Ayerst Laboratories. The L1210 mouse leukemia cells were kindly provided by Dr. V. Bono, Jr, of the NCI and the RPMI-1630 medium was supplied by the NIH Media Unit. All media were supplemented with 16.7% fetal calf serum (Flow Laboratories), which had been inactivated at 56°C for 30 minutes, and gentamicin (40 μg/ml; Schering). Cell number was estimated with a Coulter counter. Corning flasks (25 cm^2) were used for incubations.

Chlorambucil was freshly prepared in 95% ethanol to give a 50 mM stock solution. Ethanol concentrations in incubation mixtures did not exceed 0.1% vol/vol. Chlorambucil labeled with ^{14}C in the chloroethyl moiety and having a specific activity of 34.3 microcuries/mg was used for ultrafiltration studies. It was synthesized under contract by Mr. Morris Leaffer of the Stanford Research Institute. A stock solution of 50 mM was prepared in 95% ethanol, and its cytotoxic potency remained identical to the chlorambucil obtained from Burroughs-Wellcome.

Growth Studies

Cells (8–10 × 10^5/ml) in log phase were harvested from stock cultures and washed twice with fresh medium. They were seeded at 1 × 10^5 cells/ml in growth medium at 37°C with or without CPA or CPB at concentrations as indicated in individual experiments. Chlorambucil was added 15 minutes later and the incubation was continued for 35 minutes. Cells were centrifuged at 300 × *g* for 5 minutes, washed twice with complete medium, and suspended in complete medium containing 0.1% Noble agar (DIFCO). Cell survival was assessed by colony number as demonstrated by clonal growth for 2 weeks at 37°C. The detailed procedure for clonal growth in soft agar has been described (9).

Ultrafiltration Studies

Labeled chlorambucil (25–100 μM) was added to 10 ml of complete tissue culture medium or medium containing CPA or CPB (400 μM) at 37°C in 25-mm diameter stirred cells (Millipore) fitted with Amicon XM-50 membranes which retain compounds of molecular weight > 50,000. After the mixture was stirred for 5 minutes, a 200-μl sample was taken. The chamber was then pressurized with nitrogen at 30 psi and three sequential 200-μl samples of filtrate were obtained. Chambers were decompressed and a second 200-μl sample of the retained mixture was taken. Aliquots (100 μl) of each sample were added to 10 ml of Hydromix (Yorktown Research) in Kimble scintillation vials and counted in a Packard Tri-Carb scintillation counter for 10 min-

[1]Received Mar 21, 1977; accepted June 14, 1977.

[2]Division of Cancer Treatment (DCT), National Cancer Institute (NCI), Bethesda, Md.

[3]Centrophenoxine: NSC-169411; CAS reg. No. 51–68–3; acetic acid, *p*-chlorophenoxy-, 2-(dimethylamino)ethyl ester.

p-Chlorophenoxyacetic acid: NSC-8769; CAS reg. No. 122–88–3.

[4]Clofibrate: NSC-79389; CAS reg. No. 637–07–0; propionic acid, 2-(*p*-chlorophenoxy)-2-methyl-, ethyl ester.

p-Chlorophenoxyisobutyric acid: NSC-1149; CAS reg. No. 882–09–7; propionic acid, 2-(*p*-chlorophenoxy)-2-methyl-.

[5]Chlorambucil: NSC-3088; CAS reg. No. 305–03–3; butyric acid, 4-[*p*-[bis(2-chloroethyl)amino]phenyl]-. Supplied by Burroughs-Wellcome, Research Triangle Park, NC.

*Reprint requests to: Dr. Marco Rabinovitz, DCT, NCI, Bldg 37, Rm 6B05, National Institutes of Health (NIH), Bethesda, Md 20014.

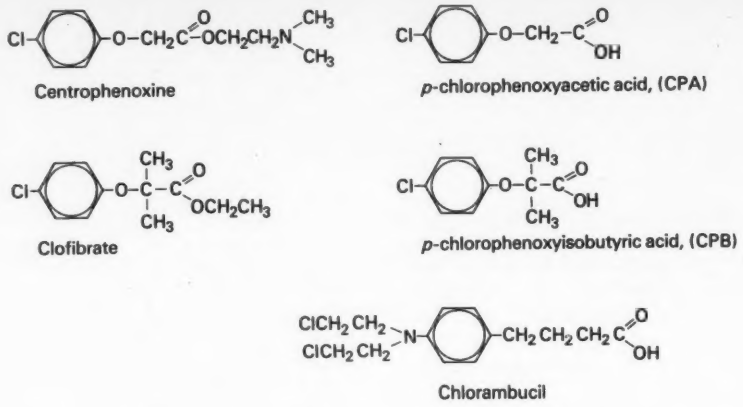


FIGURE 1.—Structures of displacing agents and chlorambucil.

utes with a counting efficiency of 75%. In each experiment the radioactivity of the three sequential samples of filtrate differed by < 5%, and that of the two samples of retained mixture differed by < 8%.

When RPMI-1630 growth medium was used without added serum, the concentration of labeled chlorambucil in the filtrate was 95% of that which remained in the chamber. However, the following membranes (designed to retain substances with molecular weights as indicated in parentheses) did *not* allow free passage of labeled chlorambucil dissolved in growth medium without serum: Amicon UM-10 (10,000) and PM-30 (30,000); Millipore PTGCO2510 (10,000) and PSEDO2510 (25,000). When serum was added to the growth medium containing CPA and CPB and the latter membranes were used, the concentration of chlorambucil was increased in the filtrate but the amount passing through the filter was less than when the Amicon XM-50 membrane was used. These results indicate that nonspecific binding of small molecules takes place on some membranes designed to allow their free passage.

RESULTS AND DISCUSSION

The reproductive capacity of cells first treated with CPA or CPB and then chlorambucil was reduced by greater than one order of magnitude when compared to cells exposed to chlorambucil alone (fig 2). The potentiation of chlorambucil cytotoxicity was not further increased when the concentration of CPA or CPB was raised from 400 to 800 μM.

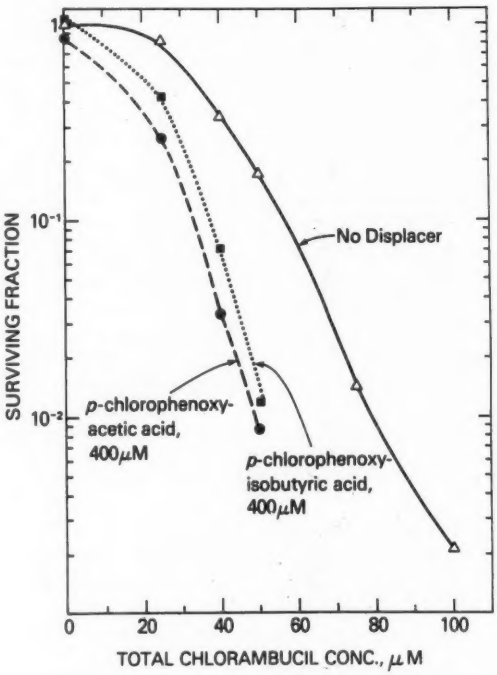


FIGURE 2.—Potentiation of chlorambucil cytotoxicity by CPA and CPB. Cells were seeded in growth medium containing CPA or CPB, chlorambucil was added, and incubation was continued for 35 mins. They were then centrifuged at 300 × *g* for 5 mins, washed twice with complete medium, and aliquots were incubated for 2 wks in nutrient-agar for estimation of clonal growth.

Ultrafiltration studies indicated that CPA or CPB nearly doubled the concentration of free chlorambucil for each total concentration of the alkylating agent tested (fig 3), and that it was the concentration of free chlorambucil which determined the degree of cytotoxicity whether or not the displacing agent was present (fig 4).

Administration of displacing agents such as CPA or CPB or their respective esterified derivatives, centrophenoxine and clofibrate (fig 1), appears to be a useful adjunct for chemotherapy with alkylating agents since a greater portion of alkylating agent is made immediately available for action at target sites. In this way, drug doses more closely represent chemotherapeutic "pulses" which might increase the therapeutic index by avoiding complications of persistence of highly toxic agents. Our results also suggest that patients receiving clofibrate (Atromid-S, Ayerst) or related compounds might require alteration of the standard chlorambucil dosage.

REFERENCES

1. KANZAWA F, HOSHI A, and KURETANI K. Enhancement of antitumor effect of cyclophosphamide by centrophenoxine. Gann 62:235–236, 1971.

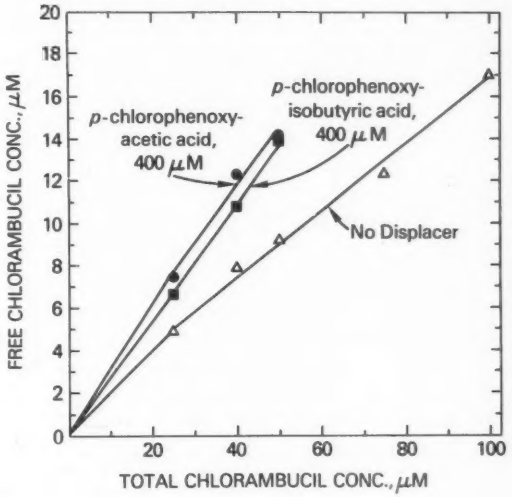


FIGURE 3.—Displacement of ^{14}C-chlorambucil from serum by CPA and CPB. Labeled chlorambucil was added to growth medium with or without CPA or CPB in ultrafiltration cells fitted with membranes designed to exclude compounds of molecular weight > 50,000. A sample of this mixture was taken and the chambers were pressurized. Sequential samples of filtrate and retained mixture were counted in a scintillation counter as indicated in the Materials section.

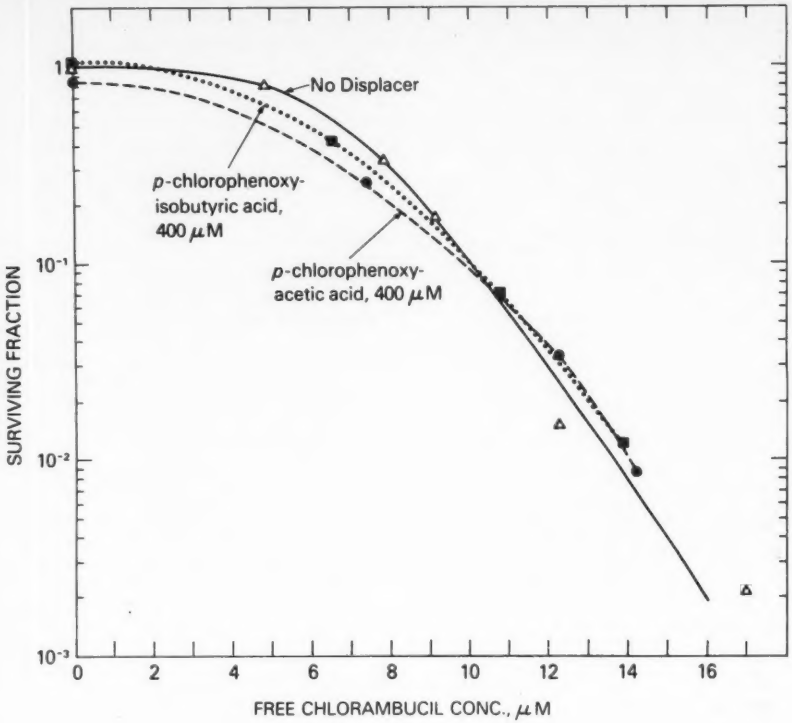


FIGURE 4.—Free chlorambucil determines cell kill. This figure was constructed from data of figs 2 and 3.

2. KANZAWA F, HOSHI A, TSUDA S, ET AL. Potentiation of antitumor activity of cyclophosphamide by centrophenoxine. Gann 63:409–413, 1972.
3. SLADEK NE. Potentiation of the antitumor effect of several nitrogen mustard analogs by centrophenoxine. Proc Am Assoc Cancer Res and ASCO 15:81, 1974.
4. SLADEK NE. Cytotoxic activity of antitumor drugs in the presence of centrophenoxine and its hydrolysis products. (Abstr) Pharmacologist 16:253, 1974.
5. GOLD J. Enhancement by clofibrate of in vivo effectiveness of cytotoxic and other chemotherapy in Walker 256 carcinosarcoma. Proc Am Assoc Cancer Res and ASCO 17:6, 1976.
6. SPECTOR AA, and SOBOROFF JM. Effect of chlorophenoxyisobutyrate on free fatty acid utilization by mammalian cells. Proc Soc Exp Biol Med 137:945–947, 1971.
7. MEISNER H. Displacement of palmitate from albumin by chlorophenoxyisobutyrate. Biochem Biophys Res Commun 66:1134–1140, 1975.
8. LINFORD JH. Some interactions of nitrogen mustards with constituents of human blood serum. Biochem Pharmacol 8:343–357, 1961.
9. SCHIFFMAN FJ, FISHER JM, and RABINOVITZ M. Ergosterol-conferred sensitivity to amphotericin B in cultured L1210 mouse leukemia cells. Biochem Pharmacol 26:177–180, 1977.

LETTERS

Lack of Severe Hypotension With VP-16–213 Administered Directly Iv[1]

Nissen et al (1) stated recently: "For iv administration of VP-16–213, a slow infusion over a ½–1-hour period is required due to the local drug toxicity." In fact, the "slow infusion route" for the epipodophyllotoxins VM-26 and VP-16–213 was prompted by earlier anecdotal reports (2,3) of immediate hypotension following their administration. Since drink ampules are unpalatable and the intestinal absorption of capsules is too erratic (4), the iv route remains important. We therefore decided to assess the safety of iv push injections as a clearly time-sparing measure.

Each 5-ml vial containing 100 mg of VP-16–213 was diluted with 5 ml of saline. With the patient lying on an examining table, the doses were injected within 3–4 minutes. The blood pressure was measured before, during, and for at least 10 minutes after the injection. The last control was done with the patient standing. If blood pressure changes were observed, a control in the erect position was done immediately after these resolved. The presence of postural hypotension was not tested for in these patients. Blood pressure changes of < 10 mm Hg were disregarded. Eighteen patients (mean age, 56.5 years; range, 31–70 years) with various malignant diseases were treated. The dosage of VP-16–213 was between 100 and 250 mg/m^2 given once weekly.

Twelve patients (47 injections) showed no blood pressure change. In six patients, some modifications of the blood pressure were noted during 11 injections; there were four rises and seven drops (another 20 injections did not produce any blood pressure change). The falls were mainly systolic (mean, 20 mm Hg; range, 15–30 mm Hg), whereas the diastolic pressure showed, if at all, only minimal modifications. All changes subsided within a few minutes and the blood pressure remained stable in the final control in the erect position.

On only one occasion was a related subjective symptom (flushing) recorded. Local vein pain was observed twice. No correlation was found between the occurrence of hypotension and the dose of VP-16–213. There does, however, seem to be a correlation with age; all patients showing blood pressure changes were > 57 years.

We feel that VP-16–213 administered directly iv is a feasible procedure and significantly facilitates the use of this drug in ambulatory patients. Caution may be necessary only in elderly patients. Furthermore, our experience has shown that patients having hypotension with VP-16–213 may be safely allowed to stand after the pressure has returned to normal in the supine position.

[1]VP-16–213: NSC-141540; 4'-demethylepipodophyllotoxin, 9-(4,6-*O*-ethylidene-β-D-glucopyranoside).

REFERENCES

1. NISSEN NI, DOMBERNOWSKY P, HANSEN HH, ET AL. Phase I clinical trial of an oral solution of VP-16–213. Cancer Treat Rep 60:943–945, 1976.
2. CREAVEN PJ, NEWMAN SJ, SELAWRY OS, ET AL. Phase I clinical trial of weekly administration of 4'-demethylepipodophyllotoxin 9-(4,6-*O*-ethylidene-β-D-glucopyranoside) (NSC-141540; VP-16–213). Cancer Chemother Rep 58:901–907, 1974.
3. MUGGIA FM, SELAWRY OS, and HANSEN HH. Clinical studies with a new podophyllotoxin derivative, epipodophyllotoxin, 4'-demethyl-9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside) (NSC-122819). Cancer Chemother Rep 55:575–581, 1971.
4. BRUNNER KW, SONNTAG RW, RYSSEL HJ, ET AL. Comparison of the biologic activity of VP-16–213 given iv and orally in capsules or drink ampules. Cancer Treat Rep 60:1377–1379, 1976.

F. Cavalli
R. W. Sonntag
H. J. Ryssel
K. W. Brunner

Division of Oncology
University Clinics
Inselspital
Bern, Switzerland

March 14, 1977

Time Relationship Between Last Dose of Daunorubicin and Congestive Heart Failure[1]

Over the past 10 years the Division of Cancer Treatment of the National Cancer Institute has supplied daunorubicin to cooperative clinical groups and independent investigators. Significant morbidity and mortality due to congestive heart failure secondary to daunorubicin treatment have been

[1]Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6.

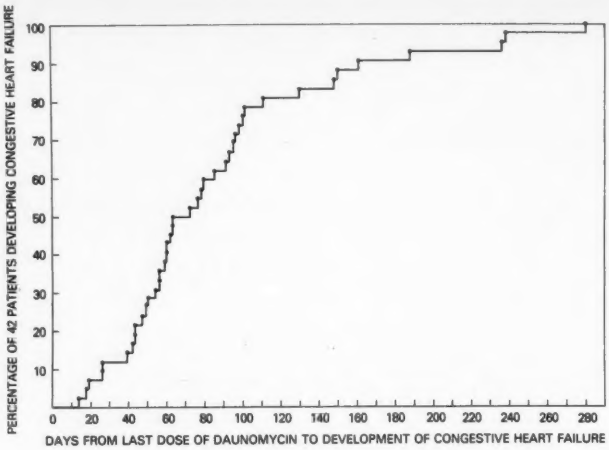


FIGURE 1.—Cumulative % of 42 patients with congestive heart failure vs time interval between last dose of daunorubicin and onset of congestive heart failure.

noted (1–3). However, the time between the last dose of daunorubicin and the onset of the cardiotoxic effect has been incompletely studied. This brief report provides data to further characterize this time interval.

A total of 5613 patients have received daunorubicin in the last 10 years, including 4760 in cooperative group studies, 394 treated by independent investigators, and 459 reported in the literature. Most of these patients had acute lymphocytic or myelocytic leukemia and received daunorubicin alone or in combination with other antileukemic agents (3).

Eighty-two cases of congestive heart failure were reported in the 5613 patients for an overall incidence of 1.46%. There were 59 cases in children (2–20 years old) and 23 in adults (> 20 years old). The data for the adult group were insufficient for our analysis, but 42 of the children had adequate flow sheets and records which formed the basis for studying the time interval in question. These children had received an average total dose of daunorubicin of 671 mg/m² (range, 270–1500 mg/m²) at different administration schedules and intervals. The mean survival time of the group was 92 days (range, 14–296 days) after the last dose of daunorubicin.

The time was recorded in days between the last dose of daunorubicin and the first clinical signs or symptoms of congestive heart failure including dyspnea, hepatomegaly, tachypnea, gallop rhythm, and cardiomegaly. In addition, the variables of age (X_1), total dose of daunorubicin (X_2), and length of time over which the drug was administered (X_3) were examined to determine their usefulness in predicting the time period (Y) between last dose and onset of congestive heart failure.

The time between the last dose of daunorubicin and the development of congestive heart failure presented a rather broad range, 14–280 days, with a mean of 89 days and a median of 63 days. Seventy-eight percent of the patients who developed heart failure did so within 101 days after the last drug dose: 60% within 79 days and 50% within 63 days (fig 1).

When the variables X_1, X_2, and X_3 were examined and considered individually, none had predictive value for Y. The correlation coefficients between the X values and Y were –0.04, 0.06, and 0.15 for X_1, X_2, and X_3, respectively, and none was statistically significant.

To determine whether the simultaneous use of X_1, X_2, and X_3 would provide a useful predictive relation, the multiple regression equation of Y on those variables was computed (4). This is the linear equation

$$Y = a+b_1X_1+b_2X_2+b_3X_3$$

which gives the best (least squares) prediction of the values of Y. A measure of the predictive usefulness of this equation is the multiple correlation coefficient, which is the simple correlation between Y and the linear combination $b_1X_1+b_2X_2+b_3X_3$. For

the daunorubicin data, the value of the multiple correlation coefficient was 0.18, which is not statistically significant. Further inspection of the data did not indicate that a nonlinear function of the X values would yield a better predictive relation.

COMMENT

The considerable range of time (14–280 days) between the last dose of daunorubicin and the onset of congestive heart failure possibly reflects the heterogeneity of the patient population, extent of disease, interval between courses of daunorubicin, and other therapy. The statistical analysis shows that age, total dose, and length of the period of administration, considered alone or in combination, are unrelated to the time between the last dose of daunorubicin and the onset of congestive heart failure. Therefore, these parameters do not provide clues to establishing the time when the patient is out of danger of developing congestive heart failure. However, the fact that cardiotoxicity may develop as long as 280 days after the last dose leads to the speculation that the short lifespan in the leukemic population may conceal a higher incidence of congestive heart failure and bias this analysis.

For the other clinically important anthracycline, adriamycin, the interval between the last dose and the onset of congestive heart failure has been reported to range from 0 to 180 days (5–8). An analysis, as outlined here for daunorubicin, is not available for adriamycin but is being undertaken by our group.

Currently there is a proliferation of anthracycline analogs with special emphasis on those with reduced cardiotoxicity. In general, most phase I trials require a minimum survival time of 60 days for patient eligibility. Our data for daunorubicin suggest that detection of only 50% of the cases of congestive heart failure secondary to an anthracycline, assuming it is as cardiotoxic as daunorubicin, would require a followup of at least 63 days after the last dose. Thus, a 60-day survival time would appear to be insufficient for evaluating the potential cardiotoxicity of an analog. Clearly, the length of observation after the last dose is an important parameter to be considered in any report discussing the incidence of congestive heart failure associated with anthracycline administration.

REFERENCES

1. HALAZUN JF, WAGNER HR, GAETA JF, ET AL. Daunorubicin cardiac toxicity in children with acute lymphocytic leukemia. Cancer 33:545–554, 1974.
2. GILLADOGA AC, MANUEL C, TAN CTC, ET AL. The cardiotoxicity of adriamycin and daunomycin in children. Cancer 37:1070–1078, 1976.
3. VON HOFF DD, ROZENCWEIG M, LAYARD M, ET AL. Daunomycin induced cardiotoxicity in children and adults: a review of 110 cases. Am J Med 62:200–208, 1977.
4. ARMITAGE P. Statistical Methods in Medical Research. New York, Wiley, 1971.
5. GOTTLIEB JA, LEFRAK EA, O'BRYAN RM, ET AL. Fatal adriamycin cardiomyopathy prevention by dose limitation. Proc Am Assoc Cancer Res 14:88, 1973.
6. MINOW RA, and GOTTLIEB JA. Adriamycin cardiotoxicity. Ann Intern Med 82:855–857, 1975.
7. LEFRAK EA, PITHA J, ROSENHEIM S, ET AL. A clinicopathologic analysis of adriamycin cardiotoxicity. Cancer 32:302–314, 1973.
8. CORTES EP, LUTMAN G, WANKA J, ET AL. Adriamycin (NSC-123127) cardiotoxicity: a clinicopathologic correlation. Cancer Chemother Rep Part 3, vol 6(2):215–225, 1975.

Daniel D. Von Hoff
Maxwell Layard
Marcel Rozencweig
Franco M. Muggia

National Cancer Institute
National Institutes of Health
Bethesda, Md 20014

March 21, 1977

Unexpected Pain Syndrome During a Phase I Study of Iv Methyl-CCNU[1,2]

Methyl-CCNU is one of several nitrosourea derivatives being evaluated for antineoplastic activity. Given orally as a single agent in doses of 175–225 mg/m^2 every 6 weeks, it results in delayed myelosuppression and frequent nausea and vomiting (1). A previously reported study using this drug iv in a 5% ethanol in 10% fat emulsion vehicle infused over 1 hour yielded moderate-to-severe delayed myelosuppression with doses of 130–150 mg/m^2 (2). Using a new solvent system consisting of a polyethoxylated vegetable oil and absolute ethanol, we conducted another phase I study.

The study included 19 patients with a variety of solid tumors. All patients had advanced disease, had had no prior nitrosourea therapy, and had recovered from all toxicity which might have resulted from prior therapy. Three or more patients

[1]Supported by contract N01-CM-43802 (Division of Cancer Treatment [DCT]), and Clinical Research Center grant 8936 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[2]Methyl-CCNU: NSC-95441; CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-.

were entered into the study at the following dose levels and schedule: 15, 30, 45, 60, 90, and 120 mg/m² of methyl-CCNU given iv every 6 weeks as tolerated. Repeat doses were allowed for patients with response or disease stabilization with dose escalations used if no significant toxicity had occurred.

The solvent system was developed in the formulation laboratory of the Drug Development Branch, Developmental Therapeutics Program, DCT, NCI (3), and consists of equal volumes of polyethoxylated vegetable oil (EML, Emulphor EL620, also used in Aqua-Mephyton Injection [GAF Corp], a non-ionic surfactant) and absolute ethanol. The methyl-CCNU powder was diluted in 4 ml of the solvent and shaken well to insure a homogenous solution which contains 25 mg/ml of methyl-CCNU.

The correct dose was then withdrawn and added to ten times the volume of sterile water. After shaking to insure a homogenous solution, the material was injected over a 20–30-minute period into the tubing of a running 5% G/W infusion. Only plastic material was used and the drug preparation was performed by a pharmacist specially trained in oncology.

RESULTS

Dose-limiting myelosuppression with methyl-CCNU used as a single agent was not achieved. Nausea and vomiting were noticed infrequently below the 60-mg/m² level and were only mild at higher doses. Patients reported a transient, pleasant state of euphoria during each injection. One patient became disoriented for 48 hours after drug administration.

Doses of methyl-CCNU of $\geq$ 90 mg/m² were associated in all but one case with an unexpected syndrome of sharp, usually severe pain in or close to the chest. The pain characteristically began 4–8 hours after injection, persisted for periods of 1–12 hours, and often required narcotics for adequate relief. One patient also had concomitant severe dyspnea with a pO_2 of 63 mm Hg. No acute EKG, chest roentgenographic, or physical changes were noted, and there were no acute changes in serum enzymes (including CPK). Only two patients receiving doses of $<$ 90 mg/m² (45 and 60 mg/m²) noted pain; they confirmed it only when specifically asked and both described it as moderate.

Two patients with stable disease were given repeat courses of iv methyl-CCNU after noting pain with the prior course; in both, the pain was exactly reproduced. In the first patient, the second dose of 90 mg/m² was dissolved in only three eights of the initial solvent volume yielding a final solvent volume equivalent to 34 mg/m² at a standard solute/solvent ratio. In these two patients, serial hourly examinations of urine sediment revealed fat droplets by Sudan stain after the onset of pain. No objective or subjective sequelae to this pain were noted, and the pain was not predictive of any beneficial tumor response.

No liver, renal, or other organ system toxic effects were noted. The study was discontinued and four patients with disease stabilization were then switched to treatment with oral methyl-CCNU.

DISCUSSION

The pain syndrome described herein remains unexplained. It was described only in or near the chest, such as in the epigastric region or the throat; in three patients it appeared to be pleuritic.

Lipuria was observed in two patients and became evident with or shortly after the onset of pain, indicating that fat embolization was occurring. Pain is not normally part of the fat embolism syndrome seen clinically after trauma (4) or lymphangiography which includes dyspnea, cyanosis, varying degrees of cerebral dysfunction, tachycardia, hypotension, petechiae, and at times, right-sided cardiac decompensation with accompanying EKG changes.

It is possible that pain-causing metabolites were concentrated in the fat droplets which subsequently became lodged in the vasculature. It is also possible that chemical modification of EML by methyl-CCNU results in altered solubility of the polyethoxylated castor oil. A separate assay for the presence of excessive plasticizer in the solution for injection after contact with a plastic gag and tubing for 1 hour was negative by the gel-permeation method.

We did not feel justified in examining further the various possibilities in man with the current solvent formulation.

REFERENCES

1. YOUNG RC, WALKER MD, CANELLOS GP, ET AL. Initial clinical trials with methyl-CCNU 1-(2-chloroethyl)-3-(4-methyl cyclohexyl)-1-nitrosourea (MeCCNU). Cancer 31:1164–1169, 1973.
2. WALKER MD, MANN-KAPLAN RS, SKLANSKY DB, ET AL. Toxicity and pharmacology of intravenous methyl CCNU (Iv-MeCCNU) in man. [Phase I]. Proc Am Assoc Cancer Res 14:39, 1973.
3. DAVIGNON JP, YANG KW, WOOD HB, JR, ET AL. Formulation of three nitrosoureas for intravenous use. Cancer Chemother Rep Part 3, vol 4(3):7–11, 1973.
4. PELTIER LF, COLLINS JA, EVARTS C, ET AL. Fat embolism. Arch Surg 109:12–16, 1974.

Charles D. Haas
Ronald L. Stephens
Carol J. Leite
J. Wendall Goodwin
Barth Hoogstraten

Division of Clinical Oncology
Kansas University Medical Center
Rainbow Blvd at 39th St
Kansas City, Kan 66103

April 25, 1977

ANNOUNCEMENTS

Medical Oncology Fellowships

Applications are now being accepted for fellowships in medical oncology at the Los Angeles County-University of Southern California Medical Center in Los Angeles to begin July 1978 and July 1979. Candidates should be board-eligible in internal medicine. The program emphasizes experience with solid tumors as well as with hematologic neoplasia. The salary is $20,853 for the first year.

Address inquiries to: David C. Stolinsky, MD, Rm 10-421, 1200 N State St, Los Angeles, Calif 90033.

Faculty Position: Hematology/Oncology

The Section of Hematology and Oncology of the University of Arizona, College of Medicine, will have an opening available in 1978 for the appointment of a physician as an Assistant Professor of Medicine. The position is for addition to the faculty component based at the Tucson Veterans' Administration Hospital, which is a fully affiliated Dean's Committee Hospital of the University of Arizona.

Physicians with particular interest in an academic career and evidence of accomplishment in clinical trials, pharmacology, and related areas should apply. The academic appointment is with the University of Arizona, with salary support provided by the Veterans' Administration. Applicants should describe their areas of particular clinical and laboratory interest and activity, and include a copy of their curriculum vitae and bibliography, and names of three professional references.

Inquiries should be addressed to: Sydney E. Salmon, MD, Head, Section of Hematology and Oncology, University of Arizona, College of Medicine, Tucson, Ariz 85724. (The University of Arizona is an equal opportunity employer.)

Seminars in Oncology at Roswell Park Memorial Institute

Buffalo, New York

There will be a seminar on "Tumors Involving the Skin" on December 8, 1977, organized by the Department of Dermatology. The chairperson is Dr. Ole Holtermann.

There is a $10.00 registration fee. *For further information contact:* Ms. Claudia Lee, Cancer Control, Roswell Park Memorial Institute, 666 Elm St, Buffalo, NY 14263.

*U.S. GOVERNMENT PRINTING OFFICE: 1977 241-171/5 1-3

CORRECTION

The title for table 6 in the paper "Combined Chemotherapy (MOPP or ABVD)-Radiotherapy Approach in Advanced Hodgkin's Disease" by Gianni Bonadonna et al is incorrect (August 1977, page 774). It should read "Tolerance to radiotherapy" rather than "Tolerance to radiotherapy in patients with no prior radiotherapy."

If your address has changed and you wish to continue receiving this publication, please send your new address and the mailing label from this copy to:

Cancer Treatment Reports
National Cancer Institute
8300 Colesville Rd
Blair Bldg, Rm 3A05
Silver Spring MD 20910

If you do not wish to continue receiving this publication, please notify us at the address noted above and enclose the mailing label from this copy.

A publication of the National Cancer Institute

DHEW Publication No. (NIH) 77-22